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Health Assessment Document for
Tetrachloroethylene (Perchloroethylene)
Final Report

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**Health Assessment Document
for
Tetrachloroethylene
(Perchloroethylene)**

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
Research Triangle Park, NC 27711

NOTICE

This document has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

PREFACE

The Office of Health and Environmental Assessment, in consultation with an Agency work group, has prepared this health assessment to serve as a "source document" for EPA use. Originally the health assessment was developed for use by the Office of Air Quality Planning and Standards; however, at the request of the Agency Work Group on Solvents, the assessment scope was expanded to address multimedia aspects.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated, and summary/conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified. Observed effect levels and dose-response relationships are discussed, where appropriate, so that the nature of the adverse health responses are placed in perspective with observed environmental levels.

Any information regarding sources, emissions, ambient air concentrations, and public exposure has been included only to give the reader a preliminary indication of the potential presence of this substance in the ambient air. While the available information is presented as accurately as possible, it is acknowledged to be limited and dependent in some instances on assumption rather than specific data. This information is not intended, nor should it be used, to support any conclusions regarding risks to public health.

If a review of the health information indicates that the Agency should consider regulatory action for this substance, a considerable effort will be undertaken to obtain appropriate information regarding sources, emissions, and ambient air concentrations. Such data will provide additional information for drawing regulatory conclusions regarding the extent and significance of public exposure to this substance.

In view of the pending release of National Toxicology Program reports on long-term animal studies with tetrachloroethylene, the carcinogenicity conclusions of this document are considered interim and will be updated when the reports can be evaluated.

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1. EXECUTIVE SUMMARY

Tetrachloroethylene (PCE) is a moderately volatile chlorinated hydrocarbon which has important commercial applications in the dry cleaning of fabrics and in the degreasing of fabricated metal parts. It is estimated that approximately 265,000 metric tons were produced in the United States in 1982. Approximately 90 percent of production is estimated to be released eventually to the atmosphere. Because PCE is relatively insoluble in water (150 mg/L) and has a vapor pressure of 19 torr at 25°C, PCE in natural waters would be conveyed to the atmosphere rapidly, through evaporation. There are no known or expected natural sources of emissions.

PCE has been detected in the ambient (natural environment) air of a variety of urban and nonurban areas of the United States and other regions of the world. Levels can range from trace amounts in rural areas to as much as 10 parts per billion (ppb) or 0.068 mg/m³ in some large urban centers. The global average background level is estimated at about 25 parts per trillion (ppt) or 0.175 x 10⁻³ mg/m³. PCE has been detected less frequently in water; it has been monitored in surface and drinking waters, generally at levels between 1 and 2 ppb. In certain instances involving contamination of groundwater, much higher levels have been reported. Although there is very limited information on the behavior of PCE in soil, PCE can be expected to leach through soils of low (< 0.1 percent) organic carbon content. The amount of PCE adsorbed to soils is dependent on the partition coefficient, the organic carbon content, and the concentration of PCE in the liquid phase.

In the troposphere, a region of the atmosphere extending to between 8 and 16 kilometers above the earth's surface, PCE undergoes photochemical degradation to the extent that its estimated lifetime is appreciably less than 1 year. Little PCE is expected to be conveyed to the stratosphere. Recent studies have shown that, in real atmospheres, neither atomic chlorine- nor hydroxy radical-induced photooxidation of PCE generates substantial concentrations of ozone or other oxidants; thus, PCE is not believed to be a significant factor in production of photochemically induced pollution often experienced near large urban centers. Because of the reduced solar flux in winter and seasonal variations in hydroxy radical concentration, PCE levels in ambient air are expected to be higher in winter than in summer. On a daily

basis, PCE levels fluctuate considerably.

Inhalation is the principal route of concern by which PCE enters the body. Ingestion of drinking water contaminated by PCE is another important concern. PCE is rapidly and virtually completely absorbed following oral administration, while pulmonary uptake of PCE during inhalation exposure is linearly proportional to exposure duration and the air concentration; pulmonary uptake is also influenced by physical activity and body mass. The metabolism and pharmacokinetics of PCE are highly contingent on its physicochemical properties. Controlled inhalation studies with human volunteers (at 100 ppm) suggest that whole-body, steady-state conditions are not established within short exposure periods (e.g., 8 hours). Because partitioning into adipose tissue is slow, steady-state (the rate at which whole body uptake is balanced by clearance) may require considerably longer periods of exposure. PCE distributes widely into body tissues. PCE is eliminated from the body mainly by the pulmonary excretion of unchanged parent compound. Limited metabolism of PCE occurs; metabolism is dose-dependent and saturable in mice, rats, and humans; in humans saturation would not be expected until air exposure levels approximate 100 ppm (678 mg/m³) or greater. The principal site of metabolism is in the liver where PCE is oxidized to PCE oxide, which rearranges to trichloroacetic acid. Controlled studies with humans have demonstrated that PCE metabolism (urinary trichloroacetic acid) represents 1 to 3 percent of the amount of PCE absorbed during 8-hour exposures to between 100 to 400 ppm (678 to 2,112 mg/m³). While the metabolic profiles of PCE are not as yet fully established in mice, rats, and humans, there is no convincing evidence of differences in the pathways in these species. PCE metabolites are known to covalently bind, in vitro and in vivo, to cellular macromolecules such as protein and lipid. Since tissue-bound metabolites have a slow rate of turnover, cumulative cellular changes may occur in humans with chronic exposure. Indices of hepatotoxicity of PCE in the rat and mouse have been shown to be highly correlative with the dose-dependent nature of PCE metabolism.

Excluding carcinogenicity as an end point, toxicity testing in experimental animals, coupled with limited human data derived principally from overexposure situations, suggest that long-term exposure of humans to

environmental levels found or expected (10 ppb or less) of PCE is not likely to present a health concern.

Decrements in task performance and coordination are the first gross signs of central nervous system (CNS) and behavioral alterations observed in controlled human studies in which individuals were exposed to about 100 ppm (678 mg/m³) for up to 7 hours. More sensitive tests, however, would have to be performed to determine if PCE affects the nervous system at even lower concentrations.

Transient liver damage in humans is generally associated with short-term exposures greatly in excess of 100 ppm (678 mg/m³). In rodent species tested, intermittent or prolonged exposure to PCE has been observed to result in liver and kidney damage at levels exceeding 200 ppm (1,356 mg/m³). Since ambient air levels are generally orders of magnitude lower than that associated with liver damage, long-term exposure of humans to ambient air or water levels would not be expected to cause adverse liver or kidney effects.

Similarly, ambient air and water levels of PCE are unlikely to cause adverse effects upon the heart.

The mammalian animal tests performed to date do not indicate any significant teratogenic potential of PCE in the species tested. On this basis, there is no evidence to suggest that the conceptus is uniquely susceptible to the effects of PCE. The anatomical effects observed primarily reflect delayed development and generally can be considered reversible. It is important to note, however, that the reversible nature of an embryonic/fetal effect in one species might, in another species, be manifested in a more serious and irreversible manner. The teratogenic potential of PCE for humans is unknown.

PCE has been evaluated for its ability to cause gene mutation, chromosomal aberrations, unscheduled DNA synthesis, and mitotic recombination. These tests were conducted using bacteria, Drosophila, yeast, cultured mammalian cells, whole mammal systems, and cytogenetic analyses of exposed humans. Certain technical and commercial samples of PCE elicited increased responses in the Ames bacterial test, a yeast mitotic recombination assay, a host-mediated assay using Salmonella, and DNA repair tests. Exogenous metabolic activation was not required for detection of these increased effects. In general, the responses were weak and were observed at high concentrations that were cytotoxic; dose-dependent relationships were not established. The positive

findings may be the result of mutagenic contaminants and/or added stabilizers. Several other tests of commercial and technical samples of PCE have been reported as negative. The epoxide of PCE, which is thought to be the active biological intermediate, was found to be positive in bacterial studies.

In a gavage bioassay, PCE has induced a statistically significant increase of malignant liver tumors in both male and female B6C3F1 mice. No carcinogenic effects were observed in lifetime studies of rats exposed to PCE in gavage and inhalation bioassays; however, these latter studies had diminished sensitivity to detect a response due to excessive dose-related mortality in the gavage study and a low dose level in the inhalation study.

Intraperitoneal injection of PCE in Strain A mice did not produce an increased incidence of pulmonary adenomas; mouse skin initiation-promotion experiments also did not show a tumor response. However, because of inherent limitations in these assays, the negative results do not detract from the positive findings of liver tumors in mice.

A cohort study of dry-cleaning workers exposed to PCE showed that workers were at an elevated risk of colon cancer mortality; however, the elevated risk cannot be totally assigned to PCE since as many as one-half of the workers may have been exposed to petroleum distillates in their working history. Other studies that found an association between cancer and employment in the dry-cleaning industry did not identify the dry-cleaning solvents to which the employees were exposed.

The positive response in both male and female mice constitutes a signal that PCE and/or its reactive metabolites might be a carcinogen for humans. In terms of strength of evidence in animal test systems, the mouse bioassay constitutes limited evidence.

According to the Agency's Proposed Guidelines for Carcinogen Risk Assessment, the cancer evidence of PCE in animal test systems is limited, and the cancer evidence in epidemiologic studies is inconclusive. The overall weight-of-evidence classification for PCE would be Group C, i.e., a possible human carcinogen.

When the criteria of the International Agency for Research on Cancer (IARC) are applied, the animal data supporting the carcinogenicity of PCE is classified as limited. Because existing epidemiologic data for PCE is inconclusive, its overall IARC ranking should be Group 3, meaning that the

evidence is inadequate to evaluate the carcinogenic potential of PCE.

The NTP is completing PCE inhalation cancer bioassays for rats and mice. These findings should be available for scientific review in the fall of 1985. When the results of these bioassays are made available, consideration will be given to updating the carcinogenicity evaluation in this assessment document.

Although PCE has only limited carcinogenicity evidence, a carcinogenic potency and related upper-bound estimate of incremental lifetime cancer risk can be estimated from the NCI male and female mouse bioassay.

The development of these risk estimates is for the purpose of evaluating the "what if" question: If PCE is carcinogenic in humans, what is the possible magnitude of the public health impact? Any use of the risk estimates should include a recognition of the weight-of-evidence likelihood for the carcinogenic potential of PCE in humans. The upper-bound incremental cancer risk is calculated to be $5.0 \times 10^{-2} \text{ (mg/kg/day)}^{-1}$ for a continuous lifetime exposure to PCE, under the presumption that PCE is carcinogenic in humans. The upper-bound nature of this estimate is such that the true risk is not likely to exceed this value, and may be lower. Expressed in terms of relative potency, PCE ranks in the lowest quartile among the 54 suspect or known human carcinogens evaluated by EPA's Carcinogen Assessment Group.

2. INTRODUCTION

Tetrachloroethylene (PCE) is one member of a family of unsaturated chlorinated aliphatic compounds. Other common names/acronyms are perchloroethylene, Perk, PER, and PERC. Its synonyms include carbon dichloride, tetrachloroethene, and 1,1,2,2-tetrachloroethylene.

PCE, though a water and solid waste contaminant, is primarily of interest in ambient air exposure situations. It is released into ambient air as a result of evaporative losses during production, storage, and/or use. It is not known to be generated from natural sources. It has negligible photochemical reactivity in the troposphere and is removed by scavenging mechanisms, principally via hydroxyl radicals.

The scientific data base is limited with reference to the effects of PCE on humans. Effects on humans have generally been ascertained from studies involving individuals occupationally or accidentally exposed. During such exposures, the concentrations associated with adverse effects on human health were either unknown or far in excess of concentrations found or expected in ambient air. Controlled PCE exposure studies have been directed toward elucidating the effects on the central nervous system, effects on clinical chemistries, and pharmacokinetic parameters of PCE exposure.

Since epidemiologic studies have not been able to assess adequately the overall impact of PCE on human health, it has been necessary to rely greatly on animal studies to derive indications of potential harmful effects. Although animal data cannot always be extrapolated to humans, indications of probable or likely effects among animal species increases the confidence that similar effects may occur in humans.

This document is intended to provide an evaluation of the scientific data base concerning PCE. The publications cited in this document represent a majority of the known scientific references to PCE. Reports which had little or no bearing upon the issues discussed were not cited.

The basic literature search that supports this assessment is current up to 1984. On-going literature searches have been conducted through 1985, resulting in the inclusion of selected references in all chapters of this document.

3. GENERAL BACKGROUND INFORMATION

3.1 PHYSICAL AND CHEMICAL PROPERTIES

Tetrachloroethylene, also called PCE (1,1,2,2-tetrachloroethylene or perchloroethylene), is a colorless, heavy liquid with a chloroform-like odor. It is used as a solvent for organic substances and is commercially important as a solvent in the dry cleaning of fabrics and in the degreasing of metals. It has a molecular weight of 165.85 and is relatively insoluble in water (150 mg/L) (Handbook of Chemistry and Physics, 1976; Chemical and Process Technology Encyclopedia, 1974). Its specific gravity at 20°C is 1.624. Its CAS registry number is 127-18-4. In air, at 25°C and standard pressure, 1 part per million (ppm) is equivalent to 6.78 mg/m³.

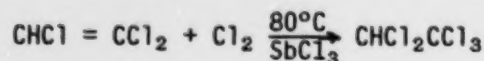
PCE has negligible photochemical reactivity (Dimitriades et al., 1983) and, in the troposphere, is decomposed via free radical mechanisms. When in contact with water for prolonged periods, PCE slowly decomposes to yield trichloroacetic and hydrochloric acids. Upon prolonged storage in light, it was reported to decompose slowly to trichloroacetyl chloride and phosgene by auto-oxidation (Hardie, 1966). At 700°C, it decomposes, when in contact with activated charcoal, to hexachloroethane and hexachlorobenzene (Gonikberg, 1956). PCE has a boiling point of 121.1°C at 760 mm Hg and a vapor pressure of 14 torr at 20°C. MacKay et al. (1982) have calculated a vapor pressure of 19 torr at 25°C.

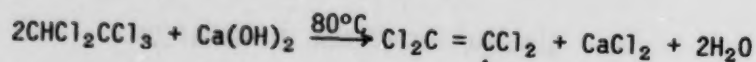
The chemical reactivity of PCE has been discussed by Bonse and Henschler (1976) in terms of the electron-inductive effect of the chlorine atoms, which reduce electron density about the ethylene bond. This effect, in combination with a steric protective effect afforded by the chlorine atoms, provides increased stability against electrophilic attack. This is exemplified in the reaction of PCE with ozone. Compared to ethylene and less-substituted chlorination hydrocarbons, PCE has a low rate of reaction (Williamson and Cvetanovik, 1968).

3.2 PRODUCTION

PCE may be produced by several processes:

1. Chlorination of trichloroethylene:

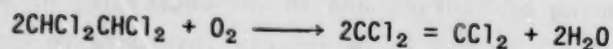




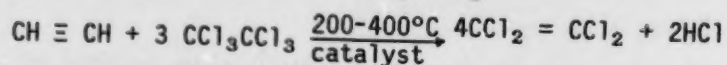
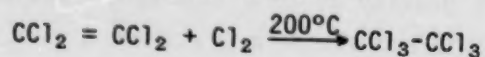
2. Dehydrochlorination of S-tetrachloroethane:



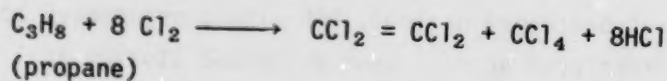
3. Oxygenation of S-tetrachloroethane:



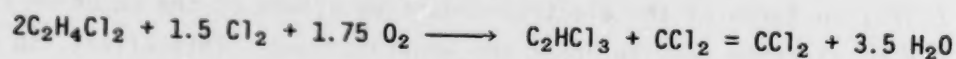
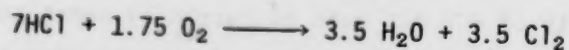
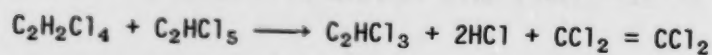
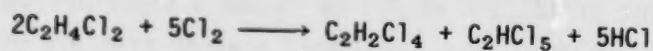
4. Chlorination of acetylene:



5. Chlorination of hydrocarbons:



6. Oxychlorination of 1,2-dichloroethane:



The majority of PCE produced in the United States is derived from the oxychlorination of 1,2-dichloroethane (reaction 6) or via chlorination of hydrocarbons (reaction 5) (Lowenheim and Moran, 1975).

In 1980, 329,000 metric tons of PCE were consumed in the United States (SRI, 1982). This figure represents production plus imports, minus exports. According to the U.S. International Trade Commission (1983), 265,770 metric tons were produced in the United States in 1982. Production in 1983 was estimated at 263,000 metric tons (Chemical and Engineering News, 1984).

TABLE 3-1. MAJOR U.S. PRODUCERS OF PCE^a

Organization	Yearly 1981 capacity, MT ^a
Dow Chemical	145
PPG	91
Vulcan	91
Diamond Shamrock	75
Ethyl Corporation ^b	
E. I. du Pont de Nemours ^c	73
Stauffer Chemical	32

^aAdapted from Chemical Economics Handbook (SRI, 1983). MT = Metric tons.

^bTerminated production (Halogenated Solvents Industry Alliance, 1983).

^cOutput for captive use only.

3.3 USE

PCE has the following uses (Gosselin et al., 1976; Fishbein, 1977): (1) dry cleaning solvent; (2) textile scouring solvent; (3) dried vegetable fumigant; (4) rug and upholstery cleaner; (5) stain, spot, lipstick, and rust remover; (6) paint remover; (7) heat transfer media ingredient; (8) chemical intermediate in the production of other organic compounds; and (9) metal degreaser.

It is estimated that the use of PCE in the dry cleaning industry represents about 42 percent of 1980 consumption in the United States (SRI, 1982).

3.4 EMISSIONS

Emissions of PCE arise during its production, from its use as a chemical intermediate in industrial processes, from storage containers, during disposal, and from its use as a solvent. Because emissions are almost exclusively to the atmosphere, the information presented in this section focuses on air. Data available concerning discharges to water are discussed in Section 3.6.1.2. Emissions estimates reflect a diversity of sources throughout the country. Dry cleaning operations are located primarily in urban areas. Approximately

26,000 establishments are estimated to exist, according to Bureau of Census data (U.S. EPA, 1979). There are approximately 18,000 retail establishments plus much smaller numbers of industrial and coin-operated facilities (International Fabricare Institute, 1984).

In 1977, global emissions were estimated at $570,000 \pm 285,000$ metric tons (Singh et al., 1979). It was also estimated that emissions accounted for approximately 90 percent of the amount of PCE produced in the United States.

3.5 ENVIRONMENTAL FATE AND TRANSPORT

The potential for ambient air and water mixing ratios of PCE to pose a hazard to human health is influenced by many processes. Such factors include transformation into secondary pollutants of concern and degradation rates in air and water.

3.5.1 Ambient Air

3.5.1.1 Tropospheric Reactivity--Reaction with the hydroxyl radical ($\cdot\text{OH}$) is the principal process by which many organic compounds, including PCE, are scavenged from the troposphere. Hydroxyl radicals are produced when O_3 is irradiated, resulting in excited atomic oxygen, which then reacts with water vapor. The tropospheric lifetime of a compound is related to the $\cdot\text{OH}$ mixing ratio according to the expression:

$$\tau_{\text{lifetime}} = \frac{1}{k [\text{OH}]} \quad (3-1)$$

where k is the rate constant of reaction.

Singh et al. (1979, 1981) calculated a tropospheric residence of PCE of about 68 days. This calculation was based on an average 24-hour $\cdot\text{OH}$ abundance of 10^6 molecules cm^{-3} in the boundary layer of a polluted atmosphere. Justification for this $\cdot\text{OH}$ mixing ratio stems from the field studies of Calvert (1976) and from Singh and coworkers (1979a). Because this $\cdot\text{OH}$ mixing ratio is more typical of summer months, Singh et al. (1981) suggested that a seasonally adjusted mixing ratio would result in a longer chemical residence time. If a seasonally averaged $\cdot\text{OH}$ mixing ratio of 4×10^5 molecules cm^{-3} (a level supported by the field measurements of Campbell et al., 1979) and a weighted global

average temperature (265°K), an average residence time of PCE would be calculated to be 292 days or 0.8 year (Singh et al., 1979).

Estimations of a residence time of PCE of one year or less have been reported by a number of investigators (Dilling, 1982; Altshuller, 1980; Singh et al., 1978a; Singh, 1977; Crutzen et al., 1978; Lillian et al., 1975; Yung et al., 1975; Pearson and McConnell, 1975).

Dimitriades et al. (1983) calculated a very low tropospheric reactivity for PCE based on observations that ambient levels of PCE are constant. Atmospheric consumption of PCE is 0.02 percent per daylight hour.

Higher levels of $\cdot\text{OH}$ have been reported for the southern hemisphere compared to those in the northern hemisphere (Singh, 1978). This gradient probably is due to the fact that carbon monoxide levels are much higher in the northern hemisphere, thus reducing $\cdot\text{OH}$ levels (Singh, 1978). Measurements of PCE and other reactive halocarbons indicate that mixing ratios are higher in the northern hemisphere where the $\cdot\text{OH}$ mixing ratio is low and where most of the PCE is released (Singh et al., 1978b).

Chamber studies indicate that PCE, on irradiation in the presence of other atmospheric constituents, can be transformed into secondary products. This area of study has been recently reviewed and further explored by Dimitriades et al. (1983). These investigators have confirmed that PCE, under smog chamber conditions having high reactant concentrations, reacts to form O_3 and ozone precursors by means of a Cl -initiated photooxidation mechanism. However, such photooxidation is not expected to occur in the real atmosphere at a rate high enough for substantial O_3 production. It is the authors' contention that Cl atoms are effectively scavenged by the hydrocarbons normally present in the atmosphere; thus, PCE was judged to contribute less to O_3 production than equal concentrations of ethane. Ethane is regarded by the authors to be a boundary species separating the reactive volatile organics from the unreactive ones.

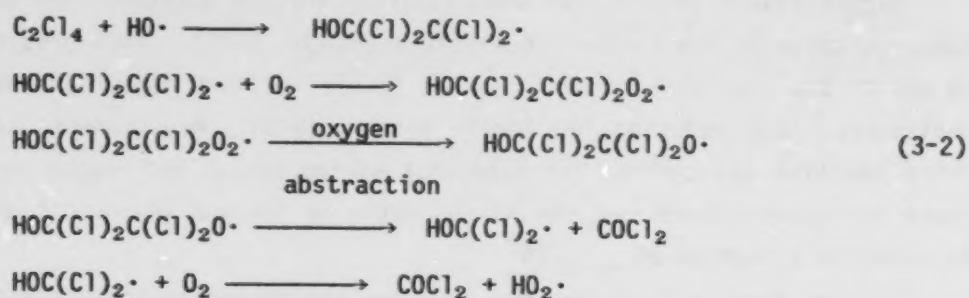
Studies on PCE reactions with O_3 , O , and $\cdot\text{OH}$ have indicated that rate constants are lower than with Cl (Dimitriades et al., 1983).

Gay et al. (1976) had determined that trichloroacetyl chloride was a photooxidation product of PCE in smog chamber studies. While the studies of Gay et al. (1976) indicate that trichloroacetyl chloride may be formed through chlorine atom migration in an epoxide intermediate, evaluation of $\cdot\text{OH}$ and oxygen atom rate constants indicate that less than 1 percent of PCE in ambient air reacts with atomic oxygen and, of the activated epoxides formed, only a small percentage undergo rearrangement (Graedel, 1978). Dimitriades et al. (1983) found

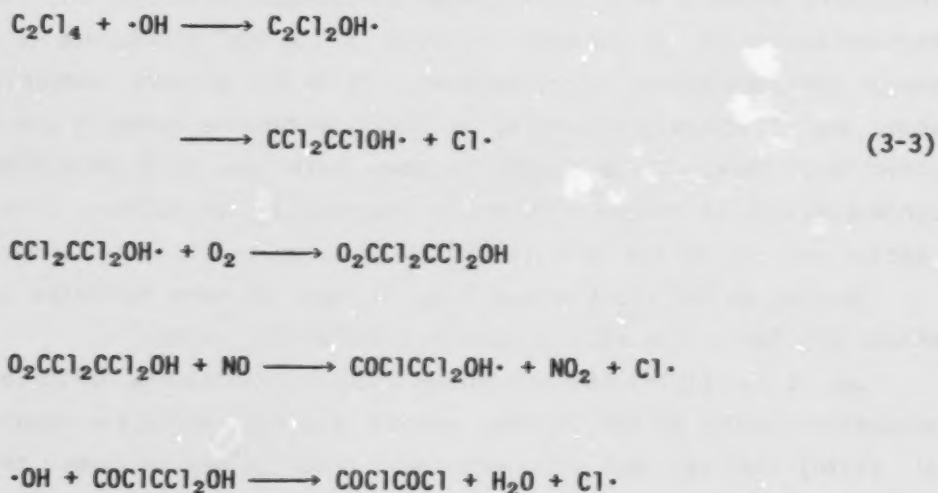
that, on irradiation, the only product observed was CO. Phosgene was not detected. When 2 ppm (13.6 mg/m³) PCE and 20 ppb trichloroacetyl chloride were irradiated together, the phosgene level reached 0.1 ppm.

Phosgene production from the photochemical oxidation of PCE in the presence of other substances has been reported by others (Lillian et al., 1975; Gay et al., 1976). The extent to which phosgene may be formed in real atmospheres, based on smog chamber results, would also be expected to be minimal.

3.5.1.2 Tropospheric Removal Mechanisms for PCE--The reaction sequence by which PCE may be scavenged from the troposphere is as follows (Graedel, 1978):



Howard (1976) suggested that the reaction path for the atmospheric oxidation of PCE may follow the scheme below, leading to the production of oxalyl chloride:



Compared to other ethylene compounds studies, Howard (1976) reported that PCE exhibits unusually low reactivity toward hydroxyl radicals.

Snelson et al. (1978) suggested that trichloroacetyl chloride and phosgene would hydrolyze to the corresponding chloroacetic acids and hydrogen chloride via homogeneous gas phase hydrolysis. The acids then would presumably be washed out of the atmosphere.

The environmental significance of the production of phosgene from PCE has been discussed by Singh and coworkers (Singh et al., 1975; Singh, 1976). As PCE emissions are likely to be higher in urban areas, the reactivity of this halocarbon may result in concentrations of phosgene in the low ppb range during adverse meteorological conditions in and around urban centers (Singh, 1976). It should be noted that overt adverse health damage would not be expected at these phosgene levels. Considering the smog chamber results of Dimitriadis et al. (1983), in which PCE was found to have negligible reactivity, it appears unlikely that phosgene would be produced at other than trace levels. Singh (1976) concluded that phosgene is removed slowly from the atmosphere. Rainfall appears to lower atmospheric levels of phosgene (Singh et al., 1977a). On the other hand, phosgene has been reported to hydrolyze to CO_2 and HCl rapidly in liquid water. Manogue (1958) reported a half-life of 0.1 second at 25°C . Thus, it is not expected to persist in the troposphere because of rainout and hydrolysis in aqueous aerosols.

The observed diurnal variations in PCE levels suggest that PCE has higher mixing ratios in the morning and evening hours than at other times (Lillian et al., 1975; Singh et al., 1977a). Ohta et al. (1977) reported that mixing ratios tended to be highest on cloudy days and lowest on rainy days.

Solar flux is a major factor in the rate at which PCE is removed from the atmosphere. Singh et al. (1977a) suggested that the reduced solar flux in winter months would permit a much longer transport of PCE because of reduced reactivity. The effect of solar flux was calculated by Altshuller (1980) who estimated that a 1 percent consumption of PCE by reaction with $\cdot\text{OH}$ would take 14 days during the month of January as opposed to one day in July.

3.5.2 Water

Jensen and Rosenberg (1975) investigated the degradability of PCE (~ 0.1 to 1.0 ppm) in both open and closed systems, with sea water and fresh water. In open aquaria, with 20 L of sea water, PCE levels decreased 50 percent within

200 hours (daylight). In a closed system, levels decreased approximately 25 percent over the same interval (daylight). It was reported that PCE levels in boiled, deionized water in a closed system did not exhibit any significant decrease after 8 days. Analysis was by headspace collection, followed by gas chromatography-electron capture detection (GC-ECD) quantification. Accuracy and limits of detection were not reported. Variation in detector sensitivity was checked daily by an injection of PCE in hexane.

Dilling et al. (1975) reported that PCE in water slowly decomposes to form trichloroacetic and hydrochloric acids. The evaporation rate was determined by dissolving 1 ppm (w/w) PCE in 200 mL of water. Solution temperature was approximately 25°C. The solution was stirred magnetically in a sealed system. Quantification was made by mass spectroscopy. The evaporation rate of PCE, determined from measurements over a 2-week period, was characterized by a 50 percent decrease in the initial mixing ratio in 24 to 28 minutes. The stirring speed had a marked effect on the evaporation rate. With no stirring except for 15 seconds every 5 minutes, the time required for 50 percent depletion ranged from 72 to 90 minutes. The evaporative half-life was 27 ± 3 minutes. Addition of dry, granular bentonite clay (500 ppm) appeared to increase the rate of disappearance by 33 percent at 20 minutes. However, when the clay was allowed to remain in contact with purified water for several days and then added to the solution, there was no change in the rate compared with control. In closed-system investigations, Dilling et al. (1975) used dry, powdered dolomitic limestone, bentonite, and peat moss to determine the adsorption rate for PCE. With 500 ppm bentonite, there was a 22 percent absorption after 30 minutes. There was no further absorption. Addition of limestone resulted in a 50 percent depletion in 20 ± 2 minutes. Addition of silica sand had no effect on the disappearance rate. When 500 ppm peat moss was added, up to 0.4 ppm PCE was absorbed after 10 minutes. At longer times, no further absorption was observed. It was concluded that evaporation probably is the major pathway by which PCE is lost from water.

Parsons et al. (1984) obtained evidence that PCE can be converted to other chlorinated compounds following incubation of PCE in microcosms containing muck from an aquifer recharge basin. Results suggested that PCE can be converted to trichloroethylene, chloroethene, cis- and trans-1,2-dichloroethene, and dichloromethane (methylene chloride).

In reactivity studies, Dilling et al. (1975) found that sunlight had the greatest effect on the rate of PCE disappearance. The PCE level in water was 6 μM . Over a 12-month period, in which PCE solutions were stored in the dark as well as in the light, PCE levels decreased from 1 ppm (0 time), to 0.63 ppm (6 months), to 0.35 ppm (12 months) in samples stored in the dark. In the light-exposed solution, the level decreased to 0.52 ppm (6 months), and 0.24 ppm (12 months).

Schwarzenbach et al. (1979), in measurements of PCE levels in Lake Zurich, reported findings compatible with those of Dilling et al. (1975) in that evaporation is the dominant elimination process from surface waters. The annual release to the atmosphere was estimated by applying a steady-state mass balance model. Based on vertical concentration profiles from the lake, about 240 kg PCE was released from the central basin annually.

Wood et al. (1981) demonstrated that PCE in sediment-water samples can be degraded under anaerobic conditions. PCE was not degraded in autoclaved controls. Under experimental conditions, the resulting half-life for PCE was 34 days.

3.6 LEVELS OF EXPOSURE

3.6.1. Mixing Ratios

3.6.1.1 Ambient Air--A wide variety of halogenated aliphatic hydrocarbons, including PCE, have been detected in ambient air. Ambient measurements of PCE have been conducted in both the United States and other areas of the world. These determinations provide a basis for assessing the levels to which human populations may be exposed.

Measured ambient air concentrations differ widely and undoubtedly reflect the influences of a variety of factors, e.g., meteorological conditions, tropospheric reactivity, diurnal variations, sampling times, and source emissions.

Table 3-2 provides summary information regarding background and urban concentrations of PCE. It should be noted that, in general, these values reflect short sampling times.

Measurements of ground-level samples by Singh et al. (1978b), in both the northern and southern hemispheres, gave average background levels of 0.040 ± 0.012 ppb ($2.7 \times 10^{-4} \pm 0.08 \times 10^{-4}$ mg/m³) and 0.012 ± 0.003 ppb ($0.081 \times 10^{-3} \pm 0.02 \times 10^{-3}$ mg/m³), respectively. Globally, the average background level of

TABLE 3-2. AMBIENT AIR MIXING RATIOS OF PCE MEASURED AT SITES AROUND THE WORLD

Location	Date of Measurement	Reported Concentration, ppb (mg/m ³) Maximum	Minimum	Average	Reference
<u>Alabama^a</u>					
Birmingham	April 12-22, 1977	0.008	0	0.001 ± 0.003	Pellizzari, 1979
<u>Arizona</u>					
Grand Canyon ^a	Nov. 28-Dec. 5, 1977	0	0	0	Pellizzari, 1979
Phoenix	Apr. 23-May 6, 1979 ^b	3.696 (0.025)	0.129 (0.0008)	0.9938 ± 0.7155 (0.0067 ± 0.0048)	Singh et al., 1981
<u>California</u>					
San Bernardino Mtns.	Fall, 1972	0.09 (6.1 × 10 ⁻⁴)			Simmonds et al., 1974
Badger Pass	May 12-16, 1976	0.03 (2 × 10 ⁻⁴)			Singh et al., 1977a
Point Arena	1976	0.03 (2 × 10 ⁻⁴)			Singh et al., 1978a
Stanford Hills	Nov. 24-30, 1975	0.04 (2.6 × 10 ⁻⁴)			Singh et al., 1977b
Point Reyes	Dec. 2-12, 1975	0.043 (2.9 × 10 ⁻⁴)			Singh et al., 1977b
Dominguez	May 14, 1976			2.9	Pellizzari, 1977
El Cajon	April 9, 1975			0.31	Su and Goldberg, 1976
La Jolla	Apr 9, 1974-Jan 6, 1976	2.3	0	0.53 ± 0.63	Su and Goldberg, 1976
Los Angeles	Sept. 22, 1972-April 19, 1979	2.2	0.067	1.1 ± 0.45	Simmonds et al., 1974; Singh, 1976; Singh et al., 1977a; Su and Goldberg, 1976
Menlo Park	Nov. 24-30, 1975			0.20 ± 0.21	Singh et al., 1977a
Mill Valley	Jan. 1-27, 1977			0.065 ± 0.075	Singh et al., 1979
Mt. Cuyamaca	Mar. 15, 1975			0.22	Su and Goldberg, 1976

TABLE 3-2. (continued)

Location	Date of Measurement	Reported Concentration, ppb (mg/m ³)	Minimum	Average	Reference
<u>California</u>					
Oakland	June 30-July 8, 1979	0.64	0.12	0.31 ± 0.17	Singh et al., 1979; Singh et al., 1981
Palm Springs	May 5-11, 1976	1.1	0.12	0.28 ± 0.084	Singh et al., 1978a
Riverside	April 25, 1977- July 12, 1980	0.98	0.37	0.49 ± 0.13	Singh et al., 1979; 1980
San Jose	Aug. 21-27, 1978			1.1 ± 0.036	Singh et al., 1979
Santa Monica	April 6, 1974			2.3	Su and Goldberg, 1976
Upland	Aug. 13-Sept. 23, 1977	1.1	0.01	0.19 ± 0.35	Pellizzari, 1979
<u>Colorado^a</u>					
Denver	Jun 16-26, 1980	0.47	0.24	0.39 ± 0.077	Singh et al., 1980
<u>Delaware</u>					
Delaware City	July 8-10, 1974	0.51 (0.0034)	<0.02 (<0.0001)	0.24 (0.0016)	Lillian et al., 1975
<u>Louisiana</u>					
Baton Rouge, Geismar, and Plaquemine	Fall, 1978	0.18 (0.001)	.001 (0.007 x 10 ⁻³)	0.017 (0.118 x 10 ⁻³)	Pellizzari et al., 1979b
<u>Maryland</u>					
Baltimore	July 11-12, 1974	0.29 (0.0019)	<0.02 (<0.0001)	0.18 (0.0012)	Lillian et al., 1975
<u>Michigan</u>					
Detroit	Oct 27-Nov 5, 1978 ^b	2.16 (0.004)	<0.1 (<0.001)	0.35 (0.002)	Evans et al., 1979
<u>New Jersey</u>					
Bayonne	March, 1973-Dec., 1973	8.2 (0.0055)	0.30 (0.0020)	1.63 (0.0110)	Lillian et al., 1975

TABLE 3-2. (continued)

Location	Date of Measurement	Reported Concentration, ppb (ng/m ³) Maximum	Minimum	Average	Reference
New Jersey					
New Brunswick	-	-	0.5 (0.003)	-	Lillian et al., 1976
New Brunswick	-	-	0.12 (0.0081)	-	Lillian and Singh, 1974
Seagirt	June 18-19, 1974	0.88 (0.059)	0.10 (0.067)	0.32 (0.0022)	Lillian et al., 1975
Sandy Hook	July 2, 1974	1.4 (95 x 10 ⁻⁴)	0.15 (10 x 10 ⁻⁴)		Lillian et al., 1975
Boundbrook, Railway, Edison and Passaic	Sept 18-22, 1978	58 (0.394) ^c	trace	30.9 (0.210)	Pellizzari et al., 1979
Batsto ^a	Feb. 26-Dec. 29, 1979	0.53	0	0.034	Bozzelli et al., 1980
Bridgeport ^a	Sept. 22, 1977	0.041	0	0.020	Pellizzari and Bunch, 1979
Burlington ^a	Sept. 19, 1977			0.027	Pellizzari and Bunch, 1979
Camden ^a	April 3-Oct. 24, 1979	30	0	1.8 ± 6.2	Bozzelli et al., 1980
Carlstadt ^a	Sept. 28-30, 1978	5.5	1.1	3.5 ± 2.3	Pellizzari et al., 1979
Edison ^a	March 24, 1976- Sept. 24, 1978	8.7	0.11	2.8 ± 3.1	Pellizzari et al., 1979; Pellizzari, 1978; Bunn et al., 1975
Elizabeth ^a	Sept. 15, 1978- Dec. 29, 1979	14	0	2.0 ± 3.1	Bozzelli et al., 1980; Pellizzari, 1979
Fords ^a	Mar. 26, 1976- Sept. 27, 1978	5.6	0	2.8 ± 2.7	Pellizzari et al., 1979; Pellizzari, 1977
Middlesex ^a	July 23-28, 1978	0.21	0	0.068 ± 0.090	Bozzelli and Kabbekus, 1979
Newark ^a	Mar. 23, 1976- Dec. 29, 1979	32	0	1.3 ± 3.1	Bozzelli and Kabbekus, 1979; Bozzelli et al., 1980; Pellizzari, 1977

TABLE 3-2. (continued)

Location	Date of Measurement	Reported Concentration, ppb (ng/m ³)	Minimum	Average	Reference
<u>New Jersey</u>					
Rahway ^a	Sept. 20-22, 1978	5.0	2.7	3.8 ± 1.2	Bozzelli and Kebbekus, 1979; Bozzelli et al., 1980
Rutherford ^a	May 1, 1978-Dec 29, 1979	9.2	0	0.89 ± 0.14	Bozzelli et al., 1980
Somerset ^a	July 18-26, 1978	0.068	0	0.036 ± 0.26	Bozzelli and Kebbekus, 1979
South Amboy	Jan. 27-Dec. 29, 1979	2.2	0	0.21 ± 0.53	Bozzelli et al., 1980
<u>New York</u>					
New York City	June 27-28, 1978 Aug 18-27, 1978 ^b	9.75 (0.0661) 10.61 (0.0721)	1.0 (0.006) 0.16 (0.001)	4.5 (0.030) 1.00 (0.006)	Lillian et al., 1975 Evans et al., 1979
Niagara Falls and Buffalo	Fall 1978	2.0 (0.014)	0.02 (0.122 × 10 ⁻³)	1.0 (0.0068)	Pellizzari et al., 1979 ^b
Whiteface Mtn.	Sept. 17, 1974	0.19 (12.8 × 10 ⁻⁴)	0.02 (0.13 × 10 ⁻³)		Lillian et al., 1975
<u>Ohio^a</u>					
Wilmington	July 16-26, 1974			0.15 ± 0.015	Lillian et al., 1975
<u>Texas</u>					
Houston	Sept 16-25, 1978 ^b	4.52 (0.030)	<0.1 (<0.001)	0.11 (0.001)	Evans et al., 1979
Aldine ^a	June 22-Oct. 20, 1977	0.037	0	0.012	Pellizzari et al., 1979
Deer Park ^a	July 29-30, 1976	0.15	0.01	0.07	Pellizzari et al., 1979
El Paso ^a	April 5-May 1, 1978	0.39	0.11	0.15	Pellizzari, 1979
Fresport ^a	Aug. 9, 1976			0.12	Pellizzari et al., 1979
Houston ^a	July 27, 1976-May 24, 1980	1.3	0	0.33	Pellizzari et al., 1979; Singh et al., 1980

TABLE 3-2. (continued)

Location	Date of Measurement	Reported Concentration, ppb (mg/m ³) Maximum	Minimum	Average	Reference
<u>Texas</u>					
LaPorte ^a	Aug. 12-13, 1976			0.49	Pellizzari et al., 1979
Pasadena ^a	July 28, 1976			0.003	Pellizzari et al., 1979
<u>Utah^a</u>					
Magna	Oct. 24-Nov. 3, 1977	0.012	0	0.004 ± 0.006	Pellizzari, 1979
<u>Washington^a</u>					
Auburn	Jan. 10-11, 1977	0.76	0.18	0.59 ± 0.27	Battelle, 1977
<u>West Virginia^a</u>					
Charleston	Sept. 27-Nov. 20, 1977	0.16	0	0.004 ± 0.008	Pellizzari, 1978
St. Albans	Sept. 27-Oct. 25, 1977	0.064	0	0.016 ± 0.032	Pellizzari, 1978

^aData obtained from summary report of Brodzinsky and Singh, 1982. Values reported are 24-hour sampling concentrations.

^b24-hour value.

^cSampling time: 14 minutes.

^dSampling time: 100 minutes.

PCE was $0.026 \pm 0.007.7$ ppb ($1.7 \times 10^{-4} \pm 0.47 \times 10^{-4}$ mg/m³); the coefficient of variation was 27 percent. The average urban level of PCE was found to be about 0.8 ppb.

Evidence for considerable variability in ambient air levels of PCE was shown by Lillian et al. (1975). The authors attributed the variability of PCE to its tropospheric reactivity (reaction with hydroxyl radicals).

Some of the highest air levels of PCE reported have been associated with waste disposal sites. Pellizzari (1978) reported levels to ranges at sites in New Jersey that ranged from trace amounts to a maximum of 58 ppb (0.394 mg/m³) in a 14-minute sampling period. PCE was adsorbed using Tenax cartridges. However, Tenax is reported to generate artifacts (Singh, 1982).

Coefficients of variation for most of the recent studies reported by Singh et al. have been less than 30 percent.

Howie (1980) reported ambient air levels of PCE in the vicinity of laundries to be as high as 32 ppb (0.22 mg/m³). In this study of indoor PCE concentrations, outdoor samples provided background data. Measurements were made by adsorbing PCE onto charcoal filters, followed by desorption with carbon disulfide and quantification by GC-ECD and GC-MS. Outdoor samples were collected for 24 hours. Of 124 measured samples, 56 had 24-hour levels of less than 1 ppb. Replicate sample analyses were reported to give an overall precision of better than 20 percent for both indoor and outdoor samples.

3.6.1.2 Water--Various studies have shown that PCE is found in both natural and municipal waters. A review by Deinzer et al. (1978) has summarized many of the findings. Love and Eilers (1982), in their review, reported that halogenated solvents such as PCE are seldom detected in concentrations greater than a few micrograms per liter in surface waters. In the U.S. Environmental Protection Agency's STORET System for the period from August 1977 to September 1984, the mean concentration of PCE in all measured water supplies was 2 µg/L. Maximum observed concentrations were about 20 µg/L. The information was compiled from 66 measurement stations in 10 states. With respect to streams and other surface waters, analysis of data from 1,102 measurement stations in 45 states indicates that, from August 1975 to September 1984, the mean PCE concentration was 1 µg/L.

3.6.1.2.1 Natural waters. Surface waters, such as rivers and lakes, are the most important sources of drinking water in the United States. Attempts have been made to show an epidemiological link between the presence of halogenated

organic compounds in drinking water and cancer (Harris and Epstein, 1976) but a cause-effect relationship has not been established.

Dowty et al. (1975a,b) detected PCE by GC-MS techniques in untreated Mississippi River water as well as in treated water. An approximate six-fold reduction in concentration occurred after sedimentation and chlorination. PCE in water from a commercial deionizing charcoal filtering unit showed a marked increase over the amount found in finished water from treatment facilities or commercial sources of bottled water. The value of charcoal filtering to remove organics from water requires further study.

Suffet et al. (1977) reported detection of PCE in river waters supplying drinking water to Philadelphia, Pennsylvania. The Belmont Water Treatment Plant, with an average capacity of 78 million gallons per day, obtains influent from the Schuylkill River.

In a study designed to detect pollutants in surface water at different U.S. sites, Ewing et al. (1977) identified PCE among the pollutants. Detection limits were not reported. The highest level reported was 45 $\mu\text{g/L}$. In all samples taken in California, Oregon, and Washington, PCE was either not detected or was found at a concentration of 1 $\mu\text{g/L}$ or less. Sampling sites included those in the vicinity of Los Angeles Harbor, Santa Monica Bay, and San Francisco Bay, at three sites along the Willamette River in Oregon, and two in the Puget Sound area.

PCE was among a number of halogenated organics found in community drinking water supply wells in Nassau County, New York. Because of contamination, 16 of these wells were closed by the New York State Health Department (Ewing et al., 1977). The maximum detected level of PCE in the contaminated wells was 375 $\mu\text{g/L}$. Since PCE is generally not used as a cesspool cleaning agent, previous industrial dumping may be the source of contamination.

Pearson and McConnell (1975) found an average PCE concentration of 0.12 ppb in Liverpool Bay sea water; the maximum concentration found was 2.6 ppb. Sediments from Liverpool Bay were found to contain 4.8 ppb (w/w). No direct correlation was found between PCE concentration in sediments and in the waters above. Rainwater collected near an organochlorine manufacturing site was found to contain 0.15 ppb (w/w) PCE (Pearson and McConnell, 1975); it was not detected in well waters. Upland waters of two rivers in Wales were found to contain approximately 0.15 ppb PCE; similar levels of trichloroethylene were found (Pearson and McConnell, 1975).

Löchner (1976) found that levels of PCE in Bavarian lake waters ranged from 0.015 to 3 ppb (0.015×10^{-3} to 2.7×10^{-3} mg/L). European surface waters were reported to have uniform PCE concentrations ranging from 0.2×10^{-3} to 0.002 mg/L. Analyses of river, canal, and sea water, all containing effluent from production and user sites in four countries, revealed PCE concentrations ranging from 0.01 to 46 ppb (0.01 to 46 µg/liter) (Correia et al., 1977).

3.6.1.2.2 Municipal waters. Bellar et al. (1974) measured the concentration of PCE in water obtained from sewage treatment plants in several cities. Before treatment, the average PCE concentration was 6.2 µg/L. The treated water before chlorination contained 3.9 µg/L PCE. After chlorination, the effluent contained 4.2 µg/L PCE.

PCE has been detected in the drinking water of a number of U.S. cities. These include Evansville, Indiana (Keith et al., 1977); Kirkwood, Missouri (Keith et al., 1977); New Orleans, Louisiana (Dowty et al., 1975); Jefferson Parish, Louisiana (Dowty et al., 1975b); Cincinnati, Ohio (Keith et al., 1977); Miami, Florida (Keith et al., 1977); Grand Forks, North Dakota (Keith et al., 1977); Lawrence, Kansas (Keith et al., 1977); New York City (Keith et al., 1977); and Tucson, Arizona (Keith et al., 1977).

Concentrations recorded for the above cities were less than 1 µg/L. An exception was Jefferson Parish, which had a measured concentration of 5 ppb (5 µg/L). Keith et al. (1977) did not detect PCE in the drinking water of Philadelphia. PCE was found in Evansville tap water from July 1971 to December 1972. The Ohio River Basin, a heavily industrialized area, is upstream from Evansville and serves as a major source of drinking water for that community.

Dowty et al. (1975b) determined levels of PCE in the drinking water for New Orleans. Considerable variation in the relative concentrations of the various halogenated compounds was observed from day to day.

Contamination of drinking water by PCE was recently investigated by Wakeham et al. (1980). It was reported that elevated concentrations of PCE were found in drinking water transported in vinyl-coated asbestos-cement pipes in areas of the town of Falmouth, Massachusetts. PCE is used as a solvent during the application of the vinyl coating to the pipe during manufacturing. It was suggested that residual solvent leaches into the water carried in these pipes.

Using a charcoal trap with flame ionization detection, Wakeham and co-workers (1980) detected PCE levels ranging from 140 to 18,000 ppb in unflushed pipes. In other parts of the distribution system, levels were less than 2 ppb. The authors reported that vinyl-coated asbestos-cement pipe has been used in parts of the northeastern United States over the past decade in response to concerns that water carried in uncoated pipes could contain asbestos fibers.

In municipal waters supplying the cities of Liverpool, Chester, and Manchester, England, 0.38 ppm (w/w) PCE was found (Pearson and McConnell, 1975).

Munich (Germany) drinking water was analyzed by Löchner (1976). Samples taken at various sampling points and times gave a range of 1.1×10^{-3} to 2.4×10^{-3} mg/L. Raw sewage at Munich contained 0.088 mg/L PCE. On mechanical clarification, the 24-hour average concentration of PCE was 0.0068 mg/L.

3.6.1.3 Sediments--Sediment levels for PCE documented in the U.S. Environmental Protection Agency's STORET system indicate that mean levels from 1,102 measurement stations in 45 states are about 33 $\mu\text{g/kg}$ (dry weight). Maximum reported levels have been as high as 3,700 $\mu\text{g/kg}$. Measurement period covers June, 1977 to November, 1984.

3.7 ANALYTICAL METHODOLOGY

PCE has been analyzed in air and in water, as well as in biological fluids, by a variety of methods. Separation of PCE from other compounds is usually carried out by gas chromatography (GC). Quantification is usually made by either electron capture detection (ECD) or mass spectroscopy (MS). These analytical methods, GC/ECD or GC/MS, have a lower limit of detection of a few ppt.

3.7.1 Ambient Air

Because PCE levels in air are typically in the sub-ppb range, the sampling and analysis techniques have been designed to detect trace gas levels.

3.7.1.1 Sampling and Sources of Error--Because of the low levels occurring in ambient air, sampling techniques have focused on adsorption onto solids such as charcoal (Evans et al., 1979) or on concentration methods that increase the amount of PCE to above detection limits (Rasmussen et al., 1977). In the upper troposphere, PCE has been sampled by pumping air into stainless steel or glass containers until there is a positive pressure relative to the surrounding atmosphere (Singh et al., 1979).

Evans et al. (1979) sampled PCE using a method based on adsorption onto activated charcoal, followed by desorption by carbon disulfide/methanol. The precision of the analytical method, expressed as a coefficient of variation for the total measurement system (including sample collection, handling, and preparation) was reported as 16 percent. When 49 quality control samples were analyzed, the overall percent recovery from the charcoal tubes was 70.2 ± 1.7 percent. When the total measurement system was independently checked by using Tenax GC, another solid adsorbent, the paired data were correlated with a coefficient of 0.82, with the average Tenax result exceeding the average charcoal result by 21 percent. Samples were in the sub- to low-ppb range. Evans et al. (1979) reported that PCE is stable on charcoal tubes for at least one month at 0°C. The lower limit of detection for the total measurement method (to include GC/ECD) was estimated at $0.68 \mu\text{g}/\text{m}^3$ (0.1 ppb).

Pellizzari and Bunch (1979) reported the use of Tenax GC, a porous polymer based on 2,6-diphenyl-p-phenylene oxide, to adsorb PCE from ambient air. Recovery was made by thermal desorption and helium purging into a freezeout trap. The estimated detection limit, when high resolution GC/MS is used, is 0.38 ppt ($2.5 \times 10^{-6} \text{ mg}/\text{m}^3$). Accuracy of analysis was reported at ± 30 percent. Included among the inherent analytical errors were (1) the ability to accurately determine the breakthrough volume, (2) the percent recovery from the sampling cartridge after a period of storage, and (3) the reproducibility of thermal desorption from the cartridge and its introduction into the analytical system. To minimize loss of sample, cartridge samplers should be enclosed in cartridge holders and placed in a second container that can be sealed, protected from light, and stored at 0°C. The advantages reported for Tenax include (1) low water retention, (2) high thermal stability, and (3) low background levels (Pellizzari, 1974, 1975, 1977; Pellizzari et al., 1976). Singh et al. (1982) have cautioned that Tenax suffers from serious artifact problems.

Krost et al. (1982) reported an estimated detection limit of 0.3 ppt for PCE using high resolution GC/MS. The detection limit was calculated on the basis of the breakthrough volume for a known amount of Tenax GC at 10°, 21°, and 32°C. Field sampling and analysis precision of the Tenax method was found to range from ± 10 to ± 40 percent relative standard deviation for different substances when replicate field sampling cartridges were examined.

Knoll et al. (1979) reported resolution of PCE from other chlorinated hydrocarbons with Carbpak C-HT, a graphitized thermal carbon black treated with

hydrogen at 1000°C. Carbowax 20M, reacted with nitroterephthalic acid, was reported not to give good separation. Porapak T, a porous polymer based on ethylene glycol dimethacrylate, was reported to give good separation.

A freezeout concentration has been developed by Rasmussen and coworkers (1977) to determine trace levels of PCE in the presence of other compounds. The detection limit was reported at 0.2 ppt (1.36×10^{-6} mg/m³) for 500-mL aliquots of ambient air samples measured by GC coupled with EDC. When freeze-out is complete, PCE remains behind, and such gases as oxygen and nitrogen are passed through as the freezeout loop is heated. The carrier gas sweeps the contents onto the column.

Singh et al. (1979, 1982) have employed the cryogenic trapping of air containing trace levels of PCE and other compounds of interest. During sampling, traps are maintained at liquid oxygen temperature. Traps were made of stainless steel packed with a 4-inch bed of glass beads or glass wool. Aliquots are thermally desorbed and injected directly into the gas chromatograph. Both electric heating and hot water desorption techniques were found to be satisfactory.

Makide et al. (1980) employed stainless steel canisters for sampling of air containing PCE at levels of about 20 ppt. Canisters were polished electrochemically. Canisters were evaluated to 10^{-4} Pa at 200°C before sampling. The composition of the samples was reported to have remained unchanged for over a year.

Budde and Eichelberger (1979) reported that carbon adsorption methods generally have more disadvantages than those methods using porous polymers. The advantage of porous polymers coupled with thermal desorption, as contrasted with solvent desorption, is higher sensitivity, because the total sample is measured and there is no background from the solvent. However, because the total sample is measured, multiple samples must be collected to insure against accident and loss of sample, and to obtain information on the precision of the method. Tenax GC was reported to be superior to other polymers for organics analysis. Samples are taken by pulling air through glass tubes packed with Tenax GC, 60/80 mesh and supported by plugs of glass wool. After a suitable sampling period (about 2 to 4 hours in urban areas), tubes are capped and stored. Samples are thermally desorbed (250 to 270°C) for 3 minutes under a 10-mL helium flow.

Criteria for evaluating methods using solid sorbents to collect organic compounds from air have been discussed by Melcher et al. (1978). Among the factors to be considered are effects of (1) size of collection tube (2) breakthrough volume, (3) humidity, (4) temperature, (5) migration, (6) desorption efficiency, and (7) concentration.

3.7.1.2 Analysis--The sampling methods which use solid adsorbents or cryogenic techniques have the trap connected to the gas chromatograph by multiple-port gas sampling valves. With solid traps, the collected organics are quickly heated and the desorbed organics are passed through capillary columns. A number of coating materials in the capillary columns have been successfully used for separating PCE. These materials include (1) SF-96 on 100/120 mesh Chromosorb W (Cronn et al., 1977); (2) SP-2100 on 80/100 mesh Supelcoport (Singh et al., 1979); (3) 80/100 mesh Carbopak C-HT, Porapak T, and SP-2100/0.1 percent Carbowax 1500 on 100/120 mesh Supelcoport (Knoll et al., 1979). In the method used by Evans et al. (1979) in field studies, a 1.8-m glass column with a 2-mm i.d., packed with 0.1 percent SP-1000 on Carbopack C, 80/100 mesh was used to separate PCE from other organics in ambient air samples. Twenty-four-hour samples were adsorbed onto charcoal. After desorption with carbon disulfide/methanol, a 1.0- μ L aliquot was injected into the gas chromatograph. The separation conditions included an oven temperature of 125°C (all transfer lines at least 170°C), and the carrier gas was 5 percent methane in argon. Quantification was made by ECD (Nickel 63; ECD temperature of 218°C) and a standing current of 0.5 ampere. To insure that the cell was not contaminated, the sensitivity of the detector was evaluated by comparing the standing current with the pulse frequency curve.

Electron capture detection is a method of choice used by a number of investigators (Singh et al., 1977, 1979, 1982; Rasmussen et al., 1977). Singh et al. (1979) maintained the ECD at a higher temperature (325°C) than did Evans et al. (1979), because it was found that the ECD response increased with an increase in temperature. The identity of PCE was confirmed by determining its ionization efficiency as well as the EC thermal response.

More recently, Singh et al. (1982) maintained the ECD at 275°C with a carrier flow rate of 40 mL/min to analyze for PCE and 11 other halogenated organics in ambient air. Identity of PCE and other compounds was established from retention times on multiple columns, the ECD thermal response, and the ECD ionization efficiency. Lillian and Singh (1974) reported that the accuracy associated with GC-ECD measurements is 75 percent or greater with compounds

having ionization efficiencies exceeding 50 percent. Using two ECDs in series, PCE was found to have an ionization efficiency of 70 percent. In a comparison of GC-ECD with GC-MS, Cronn et al. (1976) judged GC-ECD to be superior in reproducibility for quantitating halocarbons. Of four halocarbon standards (PCE not among them) measured by GC-ECD, the coefficients of variation ranged from 1.4 to 4.3 percent, compared to a range of 4 to 19 percent when 11 halocarbon standards were measured by GC-MS. A close agreement between the levels of PCE and other halocarbons determined by GC-ECD and GC-MS on the same ambient air samples was obtained by Russell and Shadoff (1977).

GC-ECD was used by Pellizzari et al. (1979) to measure PCE in ambient air samples. Samples were adsorbed onto charcoal and desorbed with a mixture of methanol and carbon disulfide, and aliquots were separated on a 2.5-mm (i.d.) Pyrex column containing 0.2 percent Carbowax 1500 on Carbopack C. The estimated detection limit was 2.5×10^{-6} mg/m³ (0.38 ppt).

Makide et al. (1980) separated trace levels of PCE from other halogenated organics on a silicone OV-101 column (10 percent by weight coated on Chromosorb W-HP, 80-100 mesh) of 5-mm i.d. and 3 m long. Samples were transferred to the column cooled at -40°C during preconcentration. Separation was carried out by raising column temperature 5°C per minute up to 70°C. Methane was added to the carrier gas (nitrogen) to improve the signal-to-noise ratio and to stabilize the baseline. Quantification was made by a constant-current ECD. The detection limit for PCE was reported as <0.05 ppt, a level unattainable under nearly all conditions. Precision was reported to be within 2 percent.

To measure PCE at levels expected to occur in occupational air, flame ionization detection has been used. The analytical method S335, suggested by the National Institute for Occupational Safety and Health (1977) for organic solvents in air, utilizes adsorption onto charcoal, followed by desorption with carbon disulfide (CS₂). PCE is separated by GC. The method is recommended for the range 96 to 405 ppm (655 to 2749 mg/m³). The coefficient of variation for the analytical sampling method is 5.2 percent. With the method, interferences are minimal, and those that do occur can be eliminated by altering chromatographic conditions.

3.7.2 Water

3.7.2.1 Sampling--A variety of techniques and methods are commonly used to sample trace levels of PCE and other halogenated organics in water samples. Coleman et al. (1981) have reported that the Grob closed-loop-stripping technique is an excellent tool to monitor organics in water at the ppt level. It

was reported that a million-fold concentration of most low and intermediate molecular weight organics can be achieved. Quantitation is performed by spiking the initial water sample with a series of internal standards, stripping at 30°C for two hours, and by chromatographing the CS₂ extract on a wall-coated open-tubular capillary.

3.7.2.1.1 Gas purging and trapping. In this method, finely divided gas bubbles are passed through the sample, transferring the organic compounds to the gas phase. The gas is then passed through a solid adsorbent in a trap. Compounds are desorbed at elevated temperature by backflushing with a carrier gas into the gas chromatograph (Budde and Eichelberger, 1979). Since the boiling point of PCE is 121°C, Tenax GC would be an effective absorbent.

The purge and trap procedure is widely used, as is the purging device developed by Bellar and Lichtenberg (1974). For most organic compounds, detection limits as low as 1 µg/L can be obtained when GC/MS is used for analysis. Mieure (1980) reported that adding salt to the sample or increasing its temperature dramatically improves the removal of most organic compounds.

Otson and Williams (1982) have described a modified purge and trap technique for evaluation of volatile organic pollutants in water. The detection limit reported for PCE was 0.1 µg/L with ECD, and 1 µg/L with flame ionization detection (FID). Tenax GC was used as packing for the combined trap/chromatographic column.

3.7.2.1.2 Headspace analysis. This method describes static sampling of the vapor phase that is in equilibrium with the aqueous sample. The concentration in the headspace is proportional to the concentration in the water (Kepner et al., 1964). In this procedure, trace organics in the range of 10 to 100 µg/L can be sampled (Mieure, 1980). Mieure (1980) reported a detection limit for PCE (analyzed by ECD) of 0.01 µg/L. With flame ionization detection, the limit was 32 µg/L. Typically, a 1- to 2-mL sample of the headspace is removed and injected into the gas chromatograph. Headspace extraction coupled with mixed column separation and ECD analysis was reported by Castello et al. (1982) to be suitable for rapid screening of drinking water supplies.

3.7.2.1.3 Liquid/liquid extraction. Mieure (1980) reported that recovery of PCE from water spiked with 2.3 to 90 µg/L ranges from 100 to 113 percent. The precision ranges from 10 to 12 (RSD). These results were obtained from a round-robin study, by the American Society for Testing and Materials (ASTM) Committee D19 on Water, using liquid/liquid extraction. The extractant was not identified.

Budde and Eichelberger (1979) cautioned that a disadvantage to this method is that very volatile compounds may be lost during extract concentration or during solvent elution from the gas chromatograph. Methylene chloride was recommended as the only general-purpose solvent.

Sheldon and Hites (1978) used methylene chloride in a sampling procedure applied to the identification of PCE and 98 other organic compounds in river water. Grab samples were collected in amber glass bottles and samples for solvent extraction were immediately preserved by acidifying to pH 2 with hydrochloric acid and by adding 250 mL of methylene chloride. The analytical techniques used were those reported by Jungclaus et al. (1978). Solvent extraction efficiencies were not determined. While PCE was previously detected in vapor stripping analysis of prior samples, it was not detected in the water samples cited in their report.

3.7.2.2 Analysis--Schwarzenbach et al. (1979) used ECD to measure PCE levels in water samples. Volatile organics were purged and adsorbed onto charcoal. Desorption was by CS₂. Quantification was made by FID and ECD.

Dowty et al. (1975a) used Tenax GC in trapping purgeable organics from water samples. The polymer containing the trapped organics was placed in the GC injection port maintained at 200°C. Final separation was made on a glass capillary column coated with Pluronic 121. The effluent of the column was split to allow for FID and ECD.

3.7.3 Biological Media

Ramsey and Flanagan (1982) have described a gas chromatographic method reported to be suitable for analysis of PCE and other organics present in blood. Detection was both by flame ionization and ECD. Approximately 200 µL of blood or 200 mg tissue is required for analysis.

3.7.4 Calibration

Singh et al. (1982) found that primary standards of PCE in the low-ppb range could be satisfactorily calibrated using permeation tubes maintained either at 30° or 70°C. Permeation tubes were standard FEP or TFE Teflon. All permeation tubes were conditioned for two weeks or longer. Errors in the permeation rate were ± 10 percent.

3.7.5 Storage and Stability of PCE

Sampling of exhaled breath commonly is accomplished by use of Saran bags or glass pipettes. Temperature and storage time of the samples before analysis are factors to be considered in obtaining accurate data.

3.7.5.1 Glass Sampling Tubes--Evaluation of glass sampling tubes was made by Pasquini (1978). Serial alveolar breath samples were collected in the tubes and the concentrations of PCE were analyzed by a gas chromatograph equipped with a flame ionization detector. Analysis of vapor retention over 169 hours indicated that glass tubes can be acceptable containers for breath samples if precautions are taken. Moisture, temperature, and tube surface and condition can greatly alter vapor retention.

In tubes filled with breath samples taken at room temperature and also stored at room temperature, the mean percent loss of PCE was 64.8 ± 9.4 . Partitioning of PCE between the vapor and liquid states appears to be a reasonable explanation for vapor retention loss. It was shown for trichloroethylene that if storage tubes were maintained at 37°C, vapor retention was greater. It was also greater if siliconized tubes were used.

3.7.5.2 Saran, Teflon and Tedlar Containers--Saran bags as storage containers for PCE vapors have been evaluated by Desbaumes and Imhoff (1971). Although it was concluded that Saran can be an acceptable container, the diffusion rate was appreciable over a 24-hour storage period. Storage temperature was not reported.

Teflon containers were judged by Drasche et al. (1972) to be more suitable than Saran even though losses of PCE due to adherence to Teflon surfaces were appreciable. Within the first 30 minutes after introduction of a mixture (relative humidity = 45 percent) of benzene, trichloroethylene, and PCE into a Teflon bag, vapor concentrations of each dropped 40 to 60 percent. However, when the bag was heated to 100°C for 30 minutes after the mixture had been stored for 44 hours at 25°C, concentrations rose to the initial values.

Knoll et al. (1979) reported that PCE, when stored at ambient temperatures for 10 days or less in Tedlar bags, was stable. When the vapor mixture is heated to 70°C, PCE is stable for no more than 5 hours.

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4. ECOSYSTEM CONSIDERATIONS

4.1 EFFECTS ON AQUATIC ORGANISMS AND PLANTS

Tetrachloroethylene (PCE) has been tested for acute aquatic toxicity in 12 species. The information presented in this chapter presents observed levels reported to result in adverse effects under laboratory conditions. It is recognized that such parameters of toxicity are not easily extrapolated to environmental situations. Test populations themselves may not be representative of the entire species, in which susceptibility of various lifestages to the test substance may vary considerably. Guidelines for the utilization of these data in the development of criteria levels for PCE in water are discussed elsewhere (U.S. EPA, 1979).

The toxicity of PCE to fish and other aquatic organisms has been gauged principally by flow-through and static testing methods (Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975). The flow-through method exposes the organism(s) continuously to a constant concentration of PCE while oxygen is continuously replenished and waste products are removed. A static test, on the other hand, exposes the organism(s) to the added initial concentration only. Both types of tests are commonly used as initial indicators of the potential of substances to cause adverse effects.

4.1.1 Effects on Freshwater Species

Alexander et al. (1978) used both flow-through (measured) and static (unmeasured) methods to investigate the acute toxicity of four chlorinated solvents, including PCE, to adult fathead minnows (Pimephales promelas). Studies were conducted in accordance with test methods described by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975).

The static and flow-through results for the 96-hour experiments indicated that PCE was the most toxic of the solvents tested. The lethal concentration (96-hour LC_{50}) necessary to kill 50 percent of the fathead minnows in the flow-through test was 18.4 mg/L (18.4 ppm); the 95 percent confidence limits were 14.8 to 21.3 mg/L (14.8 to 21.3 ppm). In comparison, the static experiments gave a 96-hour LC_{50} of 21.4 mg/L (21.4 ppm); the 95 percent confidence limits were 16.5 to 26.4 mg/L (16.5 to 26.4 ppm). Fish affected during exposure were transferred to static freshwater aquaria at the end of exposure. Only those fish severely affected by high concentrations did not recover.

When the minnows were exposed to sublethal levels for short exposure intervals, only reversible effects were observed. Endpoints evaluated were loss of equilibrium, melanization, narcosis, and swollen, hemorrhaging gills. The effective flow-through concentration (EC_{50}) of PCE that produced one or more of these reversible effects was 14.4 mg/L (14.4 ppm).

The 96-hour LC_{50} , in a static test with the bluegill (Lepomis macrochirus), was reported as 12.9 mg/L (12.9 ppm) (U.S. EPA, 1978, 1980). The most sensitive species tested is the rainbow trout (Salmo gairdneri). The LC_{50} determined by a flow-through measured procedure was 5.28 mg/L (5.28 ppm) (U.S. EPA, 1980). With embryo-larval test procedures, a chronic value of 0.840 mg/L (0.840 ppm) was obtained by the U.S. EPA (1980) for the fathead minnow.

With the freshwater invertebrate Daphnia magna, a 48-hour EC_{50} value of 17.7 mg/L (17.7 ppm) was obtained (U.S. EPA, 1980). The midge Tanytarsus dissimilis was more resistant, with a 48-hour LC_{50} value of 30.84 mg/L (30-84 ppm) determined under static, measured conditions.

4.1.2 Effects on Aquatic Plants

As cited in the U.S. EPA Ambient Water Quality Criteria Document (U.S. EPA, 1980), no adverse effects on chlorophyll a or cell numbers of the freshwater alga Selenastrum capricornutum were observed at exposure concentrations as high as 816 mg/L (816 ppm).

For the saltwater species Skeletonema costatum a 96-hour EC_{50} of about 500 mg/L (500 ppm) was determined for effects on chlorophyll a and cell number. This alga species is more resistant than the alga Phaeodactylum tricornutum for which the EC_{50} value was determined to be 10.5 mg/L (10 ppm) (Pearson and McConnell, 1975).

4.1.3 Effects on Saltwater Species

Pearson and McConnell (1975) investigated the acute toxicity of PCE on the dab (Limanda limanda), barnacle larvae (Barnacle nauplii), and on unicellular algae (Phaeodactylum tricornutum). The LC_{50} was 5 mg/L (5 ppm) for the dab. The 48-hour LC_{50} for barnacle larvae was 3.5 mg/L (3.5 ppm).

Toxicity to the unicellular alga was assessed by measuring alterations in the uptake of carbon from atmospheric carbon dioxide during photosynthesis. Uptake of carbon dioxide was measured by the use of sodium-¹⁴C-carbonate. The EC_{50} was 10.5 mg/L (10.5 ppm).

Data collected by the U.S. Environmental Protection Agency (1980) indicate that, for mysid shrimp (Mysidopsis bahia), the LC₅₀ was 10.2 mg/L (10.2 ppm) in a 96-hour static, unmeasured procedure. Chronic testing over the life cycle of the mysid shrimp resulted in a chronic value of 0.450 mg/L (0.45 ppm) (U.S. EPA, 1980). The chronic value is 0.044 times the 96-hour LC₅₀.

4.2 BIOCONCENTRATION AND BIOACCUMULATION

An indicator of the potential for a substance to result in cumulative or chronic toxic effects in aquatic species is the bioconcentration factor (BCF). Bioconcentration refers to the increased concentration of a substance within an organism (e.g., fish) relative to the ambient water concentration under steady-state conditions. As defined by Veith et al. (1979), the bioconcentration factor (K_{bcf}) is a constant of proportionality between the concentration of the chemical in fish (C_f) and in the water (C_w). This can be more clearly expressed as

$$\frac{C_f}{C_w} = \frac{K_1}{K_2} = K_{BCF} \quad \text{at steady state.} \quad (4-1)$$

Bioaccumulation, a term often erroneously used in place of bioconcentration, can be defined as that process which includes bioconcentration and any uptake of toxic substances through consumption of one organism by another. The BCF alone, however, may not be the most useful measure of the overall fate of a substance in water or of its potential for producing toxic effects, for all chemicals.

In the absence of direct measurement, a measure commonly used to assess the degree to which a compound may be bioconcentrated is the octanol-water partition coefficient. Estimates for the octanol/water partition coefficient range from 339 to 871 (Neely et al., 1974; U.S. EPA, 1980; Chiou et al., 1977). The partition coefficient has been shown to be directly related to bioconcentration potential in fish (Neely et al., 1974). According to the American Society for Testing and Materials (ASTM), a log partition coefficient exceeding a value of three is considered an indication of a high probability of measurable bioaccumulation in aquatic species (ASTM, 1978). Compounds that exhibit a large log coefficient generally are those with low water solubility and high solubility in organic solvents. Although a compound may demonstrate

a high BCF or log partition coefficient, other environmental factors that act to reduce this potential often exist. The compound may be rapidly hydrolyzed or degraded by other mechanisms. Measurable uptake by the organism may be precluded if the tissue depuration rate for the substance is great.

With regard to PCE, the BCF was calculated to be 34 and 49 in two fish species (U.S. EPA, 1980; Neely et al., 1974). Neely et al. (1974) found that the BCF for PCE and other chemicals was linearly related to the respective partition coefficients. For PCE, the log partition coefficient was 2.88, and the BCF, determined in trout (rainbow) muscle, was 39.6 ± 5.5 . The trout were exposed to two undefined levels of PCE for an undefined period of time. The extent to which the levels approached the acute LC₅₀ level for this species or whether a steady-state was achieved was not reported. A direct measurement for BCF of 49 for the bluegill (whole body) is cited in the water quality criteria document (U.S. EPA, 1980). The log partition coefficient was 2.53. The depuration rate was rapid, with a half-life of less than one day.

Although these studies suggest that PCE does have bioconcentration potential, the extent to which this potential can be manifested in the form of adverse effects can be gauged only from the results of toxicological studies.

4.2.1 Levels of PCE in Tissues of Aquatic Species

Pearson and McConnell (1975) suggested that chronic and sublethal effects of PCE may result from exposure to low concentrations of PCE, if the halo-carbon can be bioaccumulated. As a first step in addressing the question of bioaccumulation, these investigators determined levels of PCE in a variety of invertebrate and vertebrate species (Tables 4-1 and 4-2).

Among marine invertebrates, wet tissue concentrations of PCE were found to range from 1 to 9 ppb. The highest concentration (8 to 9 ppb) found was in the crab (Cancer pagurus). Higher levels were found in marine algae (13 to 20 ppb). In tissues of fish, a range of 0.3 to 41 ppb was found. Concentrations in the livers of three species of fish were found to greatly exceed those found in the flesh. Tissue levels from all species are shown in Table 4-1. Concentrations reported for fish, fish-eating birds, and marine mammals were for selected tissues such as fish liver, sea bird eggs, and seal blubber. If the reported tissue concentrations for birds and mammals are converted to a whole-body weight basis, concentrations are much lower and closer to concentrations measured in seawater, indicating little or no bioconcentration and biomagnification (U.S. EPA, 1981).

TABLE 4-1. LEVELS OF PCE IN TISSUES OF MARINE ORGANISMS, BIRDS, AND MAMMALS

Species	Source	Tissue	Trichloro- ethylene (ppb by mass on wet tissue)	PCE (ppb by mass on wet tissue)
<u>Invertebrates</u>				
Plankton	Liverpool Bay	-	0.05 - 0.4	0.05 - 0.5
Plankton	Torbay	-	0.9	2.3
Ragworm (<u>Nereis diversicolor</u>)	Mersey Estuary	-	Not detected	2.9
Mussel (<u>Mytilus edulis</u>)	Liverpool Bay	-	4 - 11.9	1.3 - 6.4
	Firth of Forth	-	9	9
	Thames Estuary	-	8	1
Cockle (<u>Cerastoderma edule</u>)	Liverpool Bay	-	6 - 11	2 - 3
Oyster (<u>Ostrea edulis</u>)	Thames Estuary	-	2	0.5
Whelk (<u>Buccinum undatum</u>)	Thames Estuary	-	Not detected	1
Slipper limpet (<u>Crepidula fornicata</u>)	Thames Estuary	-	9	2
Crab (<u>Cancer pagurus</u>)	Tees Bay	-	2.6	2.3
	Liverpool Bay	-	10 - 12	8 - 9
	Firth of Forth	-	15	7
Shorecrab (<u>Carcinus maenus</u>)	Firth of Forth	-	12	6
Hermit crab (<u>Eupagurus bernhardus</u>)	Firth of Forth	-	15	15
	Thames Estuary	-	5	2
Shrimp (<u>Crangon crangon</u>)	Firth of Forth	-	16	3

TABLE 4-1. (continued)

Species	Source	Tissue	Trichloro- ethylene (ppb by mass on wet tissue)	PCE (ppb by mass on wet tissue)
Starfish (<u>Asterias rubens</u>)	Thames Estuary	-	5	1
Sunstar (<u>Solaster sp.</u>)	Thames Estuary	-	2	2
Sea Urchin (<u>Echinus esculentus</u>)	Thames Estuary	-	1	1
<u>Marine Algae</u>				
<u>Enteromorpha compressa</u>	Mersey Estuary	-	19 - 20	14 - 14.5
<u>Ulva lactuca</u>	Mersey Estuary	-	23	22
<u>Fucus vesiculosus</u>	Mersey Estuary	-	17 - 18	13 - 20
<u>Fucus serratus</u>	Mersey Estuary	-	22	15
<u>Fucus spiralis</u>	Mersey Estuary	-	16	13
<u>Fish</u>				
Ray (<u>Raja clavata</u>)	Liverpool Bay	flesh liver	0.8 - 5 5 - 56	0.3 - 8 14 - 41
Plaice (<u>Pleuronectes platessa</u>)	Liverpool Bay	flesh liver	0.8 - 8 16 - 20	4 - 8 11 - 28
Flounder (<u>Platyethys flesus</u>)	Liverpool Bay	flesh liver	3 2	2 1
Dab (<u>Limanda limanda</u>)	Liverpool Bay	flesh liver	3 - 5 12 - 21	1.5 - 11 15 - 30

TABLE 4-1. (continued)

Species	Source	Tissue	Trichloro- ethylene (ppb by mass on wet tissue)	PCE (ppb by mass on wet tissue)
<u>Mackere1 (Scomber scombrus)</u>	Liverpool Bay	flesh liver	5 8	1 not detected
<u>Dab (Limanda limanda)</u>	Redcar, Yorks Thames Estuary	flesh flesh	4.6 2	5.1 3
<u>Plaice (Pleuronectes platessa)</u>	Thames Estuary	flesh	3	3
<u>Sole (Solea solea)</u>	Thames Estuary	flesh guts	2 11	4 1
<u>Red gurnard (Aspitrigla cuculus)</u>	Thames Estuary	flesh guts	11 6	1 2
<u>Scad (Trachurus trachurus)</u>	Thames Estuary	flesh	2	4
<u>Pout (Trisopterus luscus)</u>	Thames Estuary	flesh	2	2
<u>Spurdog (Squalus acanthias)</u>	Thames Estuary	flesh	3	1
<u>Mackere1 (Scomber scombrus)</u>	Torbay, Devon	flesh	2.1	Not detected
<u>Clupea sprattus</u>	Torbay, Devon	flesh	3.4	1.6
<u>Cod (Gadus morrhua)</u>	Torbay, Devon	flesh Air bladder	0.8 <0.1	<0.1 3.6
<u>Sea and Freshwater Birds</u>				
<u>Gannet (Sula bassana)</u>	Irish Sea	liver eggs	4.5 - 6 9 - 17	1.5 - 3.2 4.5 - 26
<u>Shag (Phalacrocerax aristotelis)</u>	Irish Sea	eggs	2.4	1.4

TABLE 4-1. (continued)

Species	Source	Tissue	Trichloro- ethylene (ppb by mass on wet tissue)	PCE (ppb by mass on wet tissue)
Razorbill (<u>Alca torda</u>)	Irish Sea	eggs	28 - 29	32 - 39
Kittiwake (<u>Rissa tridactyla</u>)	North Sea	eggs	33	25
Swan (<u>Cygnus olor</u>)	Frodsham Marsh	liver kidney	2.1 14	1.9 6.4
Moorhen (<u>Gallinula chloropus</u>)	Merseyside	liver muscle eggs	6 2.5 6.2 - 7.8	3.1 0.7 1.3 - 2.5
Mallard (<u>Anas platyrhynchos</u>)	Merseyside	eggs	9.8 - 16	1.9 - 4.5
<u>Mammals</u>				
Grey Seal (<u>Halichoerus grypus</u>)	Farne Island	blubber liver	2.5 - 7.2 3 - 6.2	0.6 - 19 0 - 3.2
Common Shrew (<u>Sorex araneus</u>)	Frodsham Marsh	-	2.6 - 7.8	1

^aLevels for trichloroethylene included for comparative purposes.

Source: Pearson and McConnell, 1975.

TABLE 4-2. ACCUMULATION OF PCE BY DABS

Tissue	Period of Exposure (days)	Mean Exposure Concentration (ppm)	Mean Concentration in Tissue (ppm)	Accumulation Factor
flesh	3 - 35	0.3	2.8 ^a (13)	x 9
liver	3 - 35	0.3	113 (14)	x 400
flesh	3 - 35	0.03	0.16 (9)	x 5
liver	3 - 35	0.03	7.4 ^b (9)	x 200
flesh	10	0.2	1.3 (7)	x 6
liver	10	0.2	69 (7)	x 350

Numbers in parentheses are number of specimens analyzed.

^aOne fish had a flesh concentration of $29.7/10^6$ and was omitted from calculations.

^bOne fish had flesh concentration of $50.3/10^6$ and was omitted from calculations.

Source: Pearson and McConnell, 1975.

The average concentration of PCE in seawater taken from Liverpool Bay, an area where many species of organisms were collected, was 0.00012 ppm. A comparison of this value with those presented in Table 4-1 indicates an uptake of as much as 75-fold. It was the authors' contention that, based on their observations, there is little indication that bioaccumulation occurs in the food chain.

As shown in Table 4-2, dabs (*Limanda limanda*) exposed to 0.3 ppm for 3 to 35 days were found to have a BCF (liver) for PCE of 400. It was not reported whether this period of exposure approximated a steady-state for PCE. After dabs were returned to clean seawater, the level of PCE dropped to 1/100 of the original level in 4 days and to 1/1000 of the initial level after 11 days (Figure 4-1). The ratio between liver and flesh concentrations is approximately 100 to 1. The relationship between flesh and liver concentrations in the dab is shown in Figure 4-2.

Dickson and Riley (1976) detected PCE in three species of mollusks and in five species of fish collected near Port Erin, Isle of Man. Levels of PCE in various tissues are shown in Table 4-3. Relative to the PCE concentration

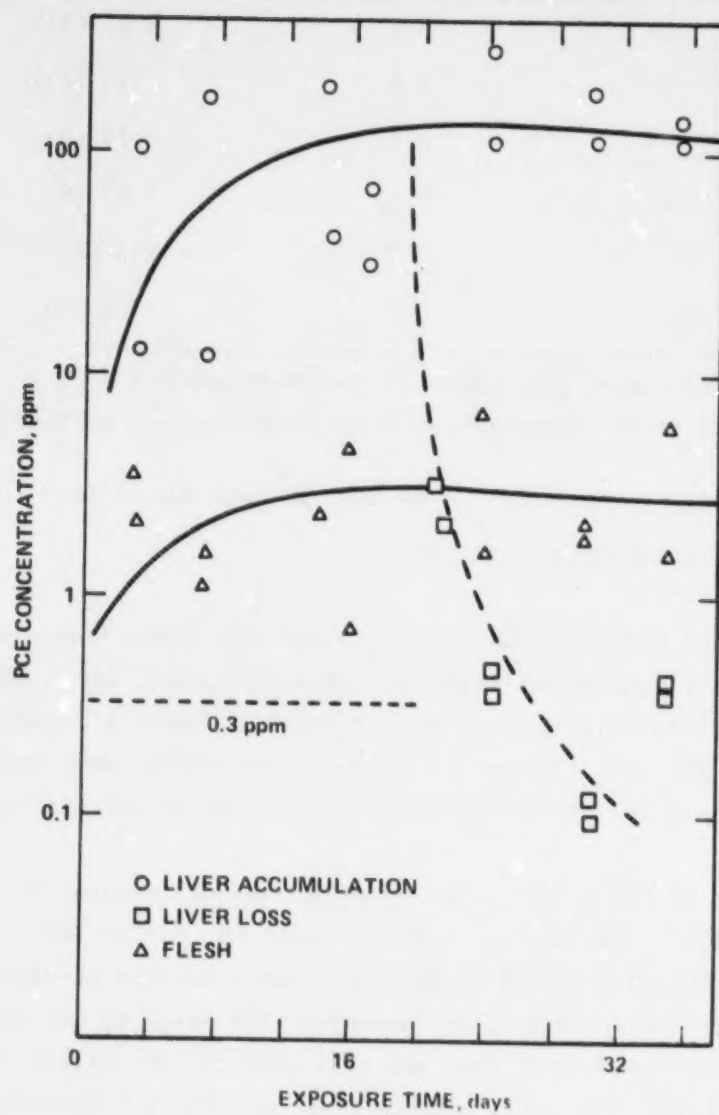


Figure 4-1. Accumulation and loss of PCE by dabs.

Source: Alexander et al., 1978.

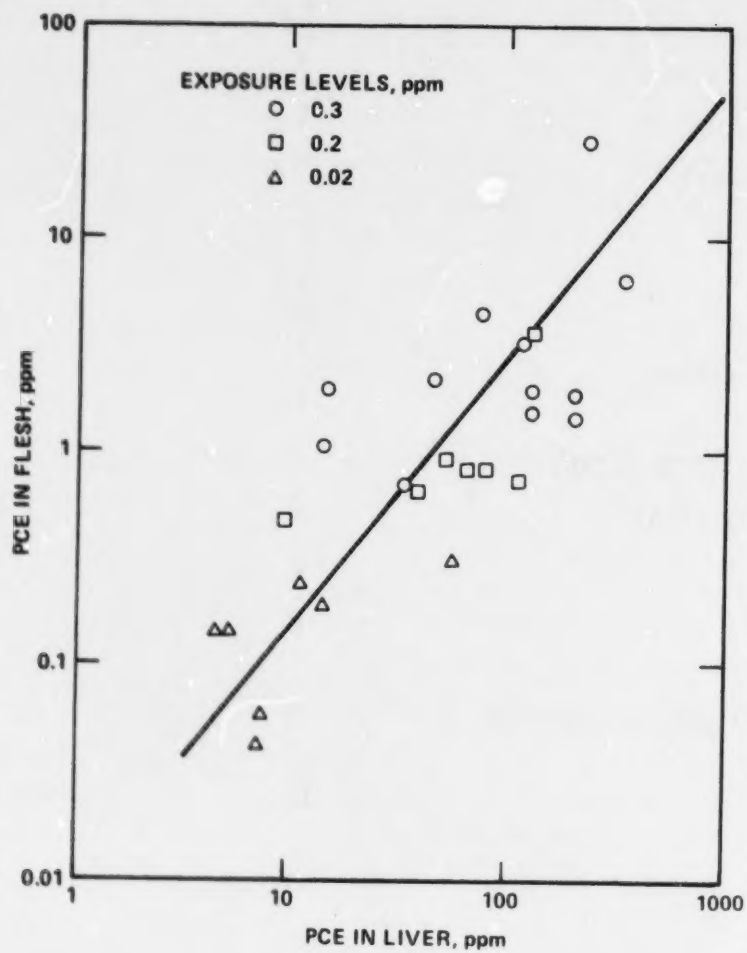


TABLE 4-3. CONCENTRATION OF PCE AND TRICHLOROETHYLENE IN MOLLUSKS AND FISH NEAR THE ISLE OF MAN

Species	PCE (mg x 10 ⁶ /g dry weight tissue)	Trichloroethylene (mg x 10 ⁶ /g dry weight tissue)
<u>Eel (Conger conger)</u>		
brain	6	62
gill	2	29
gut	3	29
liver	43	43
muscle	1	70
<u>Cod (Gadus morhua)</u>		
brain	3	56
gill	-	21
heart	3	11
liver	8	66
muscle	2	8
skeletal tissue	-	-
stomach	6	7
<u>Coalfish (Pollachius birens)</u>		
alimentary canal	-	306
brain	-	71
gill	4	-
heart	-	-
liver	6	70
muscle	2	8
<u>Dogfish (Scylliorhinus canicula)</u>		
brain	12	40
gill	13	176
gut	-	41
heart	-	274
liver	9	479
muscle	-	41
spleen	-	307
<u>Bib (Trisopterus luscus)</u>		
brain	-	-
gut	4	-
liver	0.3	143
muscle	-	187
skeletal tissue	-	185

TABLE 4-3. (continued)

Species	PCE (mg x 10 ⁶ /g dry weight tissue)	Trichloroethylene
<u>Baccinum undatum</u>		
digestive gland	33	2
muscle	39	-
<u>Modiolus modiolus</u>		
digestive tissue	-	56
mantle	63	250
muscle	16	33
<u>Pecten maximus</u>		
gill	88	detected
mantle	40	-
muscle	24	-
ovary	-	-
testis	176	-

Source: Dickson and Riley, 1976.

in seawater, there was only a slight enrichment in the tissues (< 25 times). PCE had one of the lowest mean bioconcentration factors.

4.3 BEHAVIOR IN WATER AND SOIL

The potential of any substance for bioconcentration is influenced by many factors, including the rate at which it volatilizes and its reactivity.

In the laboratory study by Dilling et al. (1975), the measured half-life of PCE ranged from 24 to 28 minutes in water. Factors affecting the evaporation of PCE were surface wind speed, agitation of the water, and water and air temperatures. Reactivity of PCE in water was measured by exposing sealed quartz tubes containing 1 ppm PCE to sunlight for one year. At 6 months, the level of PCE had declined to 0.52 ppm, and at 1 year, to 0.25 ppm. Dilling et al. (1975) reported that the presence of 3 percent NaCl (as in seawater) caused about a 10 percent decrease in the evaporation after 40 percent had already evaporated. The addition of 500 ppm clay appeared to increase the rate of disappearance to 85 percent soluble depletion at 20 minutes. These experiments were conducted to simulate the evaporation of PCE under conditions

more nearly like those found in the environment. Evaporation of PCE from the hydrosphere is a rapid process.

The field studies of Zoeteman et al. (1980) suggest that PCE is more persistent in natural water environments than is indicated by laboratory measurements. In a field study of the persistence of a variety of organic chemicals in different aquatic environments in the Netherlands, Zoeteman et al. (1980) estimated the persistence of PCE in river water from 3 to 30 days (half-life). In lakes and groundwaters, the half-life was estimated at 10-fold higher. Estimates were derived from monitored values of samples collected between two sites along the Rhine River, into which no discharges were expected. PCE was analyzed by GC-MS.

Estimates of the persistence of PCE in rivers, lakes, and ponds, by calculation according to Smith et al. (1980) are in general agreement with the field results of Zoeteman and coworkers (1980). The half-life of PCE is obtained from the expression:

$$t_{1/2} = \frac{0.693}{k_v^C}, \quad (4-2)$$

where k_v^C is the volatilization rate constant.

Using the data provided by Smith et al. (1980), the $t_{1/2}$ (days) is as follows: ponds, 9 to 20; lakes, < 1 to 20; rivers, < 1 to 20.

Bouwer et al. (1981) found that PCE and other halogenated organics have the potential to leach rapidly through soil. When secondary treated municipal wastewater containing from 1 to 10 ng/L PCE was applied to soil columns, at rates typical of high-rate land application systems and under conditions in which volatilization was prevented, PCE was detected in the effluent. Leaching of PCE through soil was suggested by Zoeteman et al. (1980) as a probable factor in the contamination of groundwater supplies in the Netherlands.

The potential for halogenated organics, including PCE, to contaminate groundwater supplies via leaching from surface waters was examined by Schwarzenbach and Westall (1981). In batch and column experiments with various types of sorbents and organics designed to simulate field conditions, these investigators found that the partition coefficient for a particular compound can be estimated from its octanol/water partition coefficient and from the fraction

of organic carbon in the sorbent. A high degree of correlation was found between the partition coefficient and organic carbon content when the fraction of organic carbon was greater than 0.1 percent. A partition coefficient of 0.56 ± 0.09 was found for PCE, using natural aquifer material (organic carbon = 0.15 percent) from a field site in Switzerland. It was concluded that, for concentrations typically encountered in natural waters, sorption of PCE and other organics of comparable lipophilicity by aquifer materials is reversible. The expression

$$S = k_p C, \quad (4-3)$$

where S = concentration in solid phase

k_p = partition coefficient

C = concentration in liquid phase

was found satisfactory to describe sorption equilibrium.

4.4 SUMMARY

The available data for PCE indicate that acute and chronic toxicity to freshwater aquatic life can occur at concentrations around 5280 and 840 $\mu\text{g/L}$, respectively. For saltwater aquatic life, the acute and chronic toxicity values are 10,200 and 450 $\mu\text{g/L}$, respectively.

PCE does not appear to biomagnify or concentrate as it moves up the food chain. The available data suggest that the bioconcentration potential of PCE is low, and it appears to be eliminated rapidly from aquatic organisms.

Contamination of groundwater supplies by PCE leaching through soil could be a concern, particularly in situations in which soils of low organic carbon content are involved.

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5. MAMMALIAN METABOLISM AND PHARMACOKINETICS

The metabolism and pharmacokinetics of PCE are highly contingent on the physicochemical properties of this compound. It is a volatile liquid at room temperature with a relatively low vapor pressure (19 torr at 25°C), is nearly insoluble in water but is highly lipophilic. Table 5-1 compares the physical properties of PCE with other familiar chloro-substituted ethylenes. It is of interest to note that with increasing chloro-substitution, water solubility decreases and lipid solubility increases. These properties determine to a considerable extent the absorption, distribution, and routes of elimination of PCE from the body, i.e., the pharmacokinetics of PCE. All chloroethylenes are thought to share as common metabolic steps in the body a) epoxidation of the ethylene double bond, b) rearrangement to the halogenated acetaldehyde or acyl halide, and c) partial transformation of the latter to a halogenated acetic acid (Bolt et al., 1982).

5.1 ABSORPTION AND DISTRIBUTION

5.1.1 Dermal Absorption

Studies have shown that absorption of PCE through the skin, from vapor exposure or from partial body immersion, is minimal in comparison to oral and inhalation routes of exposure. Riihimaki and Pfaffli (1978) exposed human volunteers (lightly clad and with respirators to prevent pulmonary absorption) to 600 ppm (4068 mg/m³) PCE for 3.5 hours and estimated percutaneous absorption of the vapor to be about 1 percent of pulmonary absorption. Stewart and Dodd (1964) and Hake and Stewart (1977) have also experimentally estimated skin absorption in volunteers by noting PCE appearance and concentration in exhaled air after immersion of the thumbs in liquid PCE. The mean peak concentration of PCE in exhaled air 40 minutes after immersion was only 0.31 ppm (2.1 mg/m³), and after 2 hours, 0.23 ppm (1.6 mg/m³), indicating that the rate of absorption through the skin is very slow, even after allowing for storage and metabolism. Jakobsen et al. (1982) experimentally quantified transport of PCE and other chlorinated hydrocarbons across guinea pig skin. Liquid contact (skin area, 3.1 cm²) was maintained for up to 6 hours and solvent concentration monitored in

TABLE 5-1. PHYSICAL PROPERTIES OF PCE
AND OTHER CHLOROETHYLENES

	Vapor Pressure at 25°C, 760 torr	Ostwald Solubility, 37°C		
		Water/ air	Blood/ air	Olive oil/ air
Vinyl chloride (1-chloroethylene)	gas	--	--	--
Vinylidene chloride (1,1 dichloroethylene)	600	--	--	69
Trans,1,2 dichloroethylene	350	2.1	5.8	189
Cis 1,2 dichloroethylene	250	2.9	9.2	270
Trichloroethylene (1,1,2 trichloroethylene)	90	1.3	9.5	718
Tetrachloroethylene (1,1,2,2 tetrachloroethylene)	19	0.43	13.1	1917

Adapted from Sata and Nakajima, 1979.

Conversion factor: 1 ppm vapor in air = 6.78 mg/m³ at 25°C, 760 torr.

blood during and following dermal applications. Blood concentrations (reflecting absorption rate) increased rapidly, peaking at 0.5 hour (1.1 µg/mL) and then decreased with time, suggesting that changes in dermal permeability occur with time. This pattern was found to be general and characteristic of chlorinated hydrocarbon solvents like PCE. Similar observations have been made by Tsuruta (1975), who estimated the percutaneous absorption rate of PCE through mouse skin at 24 nmol/min/cm² of skin. Kronevi et al. (1981) have observed histological changes in guinea pig derma within 15 minutes of topical exposure to PCE. Stewart and Dodd (1964), Hake and Stewart (1977), Morgan (1969), and Ling and Lindsay (1975) have reported severe and prompt erythema after dermal contact with PCE in man.

5.1.2 Oral Absorption

PCE is rapidly and virtually completely absorbed into the body from the gastrointestinal tract, presumably because of its high lipid solubility. Pegg et al. (1979) and Schumann et al. (1980) have administered ¹⁴C-PCE, 1 and

500 mg/kg (in corn oil) to both rats and mice and recovered better than 90 percent of the radioactivity in urine, feces, exhaled air, etc. For the rat, peak blood concentration occurred within 1 hour of oral dosing (Figure 5-1). Similar results have been found by Daniel (1963) after oral dosing of ^{36}Cl -PCE to rats (1.1 to 6.1 g/kg). Frantz and Watanabe (1983) found virtually complete absorption of ^{14}C -PCE in drinking water (saturated; approximately 150 ppm w/v; 150 $\mu\text{g/mL}$) by rats ingesting ad libitum over a 12-hr period with an average consumption equivalent to an 8 mg/kg dose.

5.1.3 Pulmonary Absorption

5.1.3.1 Man. PCE in vapor form in air is readily absorbed through the lungs into blood by first-order diffusion processes. Pulmonary uptake of a volatile compound like PCE during inhalation exposure is largely determined by the ventilation rate (about 4 to 8 L/min for man at rest), duration of exposure at a given air concentration, solubility in blood and other body tissues, and its metabolism. When body tissue concentrations (body burden) are at steady-state with inspired air concentration, the rate of uptake is equal to the rate of metabolism plus nonpulmonary excretion of PCE (i.e., by definition uptake per unit time must equal excretion by all routes at steady state). Since there are no known significant routes of excretion of PCE except pulmonary and metabolism (Section 5.2), the steady-state uptake rate approximates the metabolism rate.

The blood/gas (air) partition coefficient (λ b/g) at 37°C expresses the solubility of a solvent such as PCE as the ratio of the concentrations in blood (mg/L) and in air (mg/L). When there is no impairment of pulmonary diffusion, circulation, or ventilation, this partition coefficient exists between the arterial blood concentration (C_A) and the inspired air concentration (C_a) (or exhaled air concentration postexposure), so that $C_A/C_a = \lambda$ b/g. As illustrated in Figure 5-2, blood concentration parallels air concentration in a fixed ratio. The blood/gas partition coefficient for PCE is about 13 to 15. It is considerably higher than other solvents such as trichloroethylene (TCI), 9.5 and methylchloroform (MC), 5 (Monster, 1979; Sato and Nakajima, 1979; Table 5-1). Thus PCE has the potential for a greater pulmonary uptake rate than TCI or MC.

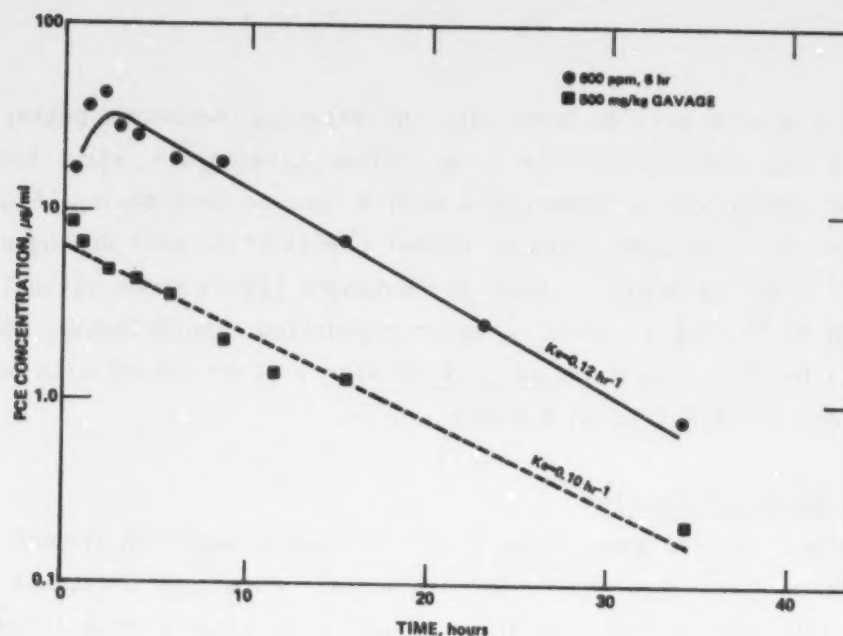


Figure 5-1. First-order excretion curves for PCE in blood of rats after exposure to 600 ppm for 6 hr (---) or to 500 mg/kg gavage doses (—). The animals were adult male Sprague-Dawley rats weighing approximately 250 g. The body burdens of PCE were about 77.5 mg per animal from inhalation exposure and 123.2 mg per animal after oral dosage. (From Pegg et al., 1979).

The rate of uptake of PCE by body tissues from blood for a given concentration in inhaled air is determined by tissue volume, the relative solubilities in blood and tissue (tissue/blood partition coefficients), and by the tissue blood flow. In accordance with the physiological model for PCE of Gubaran and Fernandez (1974), uptake by body tissues is a blood flow-dependent process which can be grouped into four physiological compartments for convenience in analysis: the blood vessel-rich group of tissues (VRG) (corresponding to brain, heart, hepatoportal system, kidneys, and endocrine glands), the muscle group (MG) (muscle and skin), the fat group (FG) (adipose tissue and yellow marrow), and the vessel-poor group (VPG), composed of connective tissue (bone, cartilage, etc.). The predicted uptake and distribution of PCE to these tissue groups during and after 8-hr exposure to 100 ppm (678 mg/m^3) is shown in Figure 5-3. For an exposure of this magnitude, body burden is calculated as about 1000 mg PCE for a 70-kg man, with half this amount distributed into fatty tissue.

With prolonged inhalation exposure to PCE, when a whole-body steady-state condition is reached with inspired air concentration, the concentration of PCE

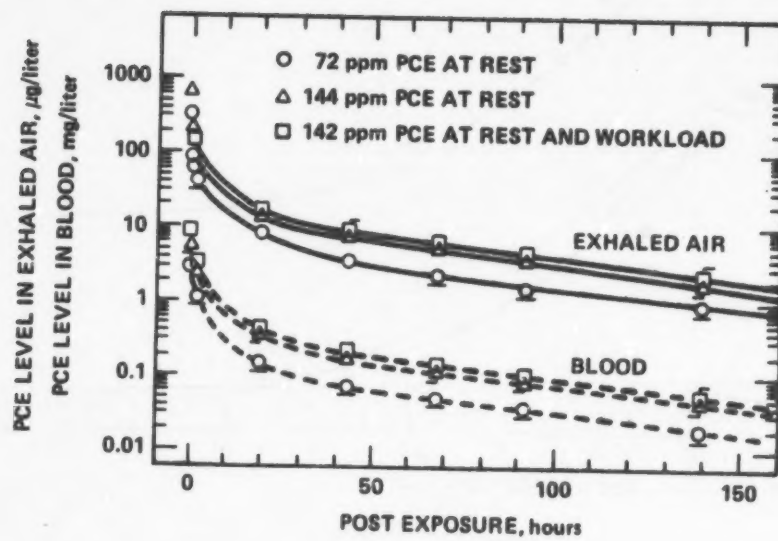


Figure 5-2. PCE concentrations in blood and exhaled air following inhalation exposure for 4 hr (means of 6 subjects). From Monster et al., 1979.

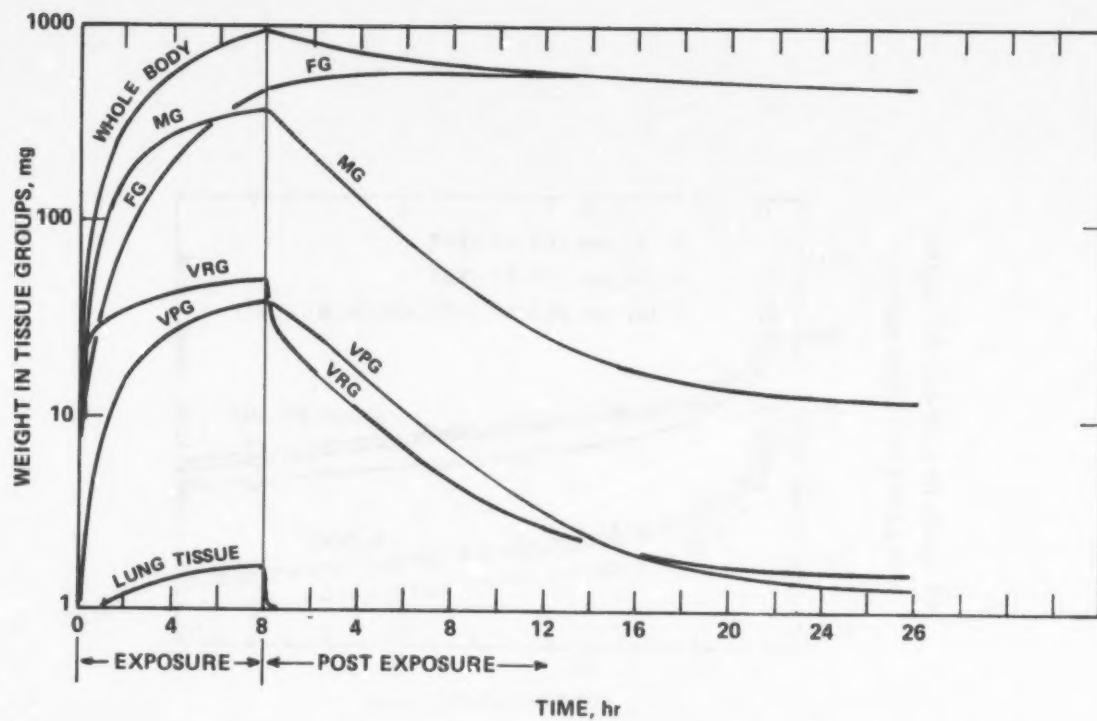


Figure 5-3. Predicted uptake and distribution of PCE to tissue groups during and after an 8-hr exposure to 100 ppm. See text for details. From Guberan and Fernandez, 1974.

in the individual tissue groups relative to blood is determined by the tissue/blood partition coefficients. For highly lipid-soluble solvents such as PCE, the adipose tissue has the highest partition coefficient and hence the highest tissue concentration at steady state. For the three solvents, PCE, TCI, and MC, the adipose tissue/blood partition coefficients at 37°C are about 105, 75, and 50, respectively (Monster, 1979; Sato and Nakajima, 1979; Table 5-1). The capacity of a tissue to take up PCE is the product of volume and solubility. Since the volume of adipose tissue in a 70-kg man is about 10L, then adipose tissue capacity for PCE is about 1500 mg; but because of the small rate of perfusion of adipose tissue (about 0.4L blood/min; Eger, 1963), the time needed to equilibrate the adipose tissue is large in comparison to that of other tissues. Figure 5-3 indicates that a steady-state plateau concentration is not achieved for adipose tissue within the period of an 8-hr exposure to PCE, although it is for muscle tissue and other vessel-rich tissues. For a given concentration in blood (or air), the half-time ($T_{1/2}$) (i.e., the time necessary to equilibrate the adipose tissue to 50 percent of its final concentration) is about 25 hours for PCE (Monster, 1979; Fernandez et al., 1976). This means that during a single 8-hr exposure, adipose tissue does not reach steady-state equilibrium, which requires about five times $T_{1/2}$, or about 125 hours.

At whole-body steady-state condition, the net pulmonary uptake of PCE is balanced with excretion of PCE by other routes, principally by metabolism. The difference between the inspired air concentration and end alveolar air concentration (exhaled air concentration) provides a measure of PCE uptake, so that:

$$Q = (C_{ins} - C_{alv}) \dot{V} \cdot T \quad (5-1)$$

where Q is the amount absorbed during time T (min), C is the air concentration in mg/L, and $\dot{V}$ is the ventilation rate in L/min. The percent retention (R) is defined as:

$$R = \frac{(C_{ins} - C_{alv})}{C_{ins}} \quad (5-2)$$

and therefore

$$Q = R (C_{ins} \cdot \dot{V} \cdot T)/100 \quad (5-3)$$

Figure 5-4 shows the change in end alveolar concentration during exposure of 5 subjects for 8 hours at 100 ppm (678 mg/m^3) (Fernandez et al., 1976). The data show a rapid rise in alveolar concentration during the first hour of exposure, followed by a slower but continuous rise during the subsequent 7 hours of exposure (retention value decreasing with exposure) towards a fixed retention value at whole-body equilibrium, although it is apparent from Figure 5-4 that equilibrium or steady-state retention is not reached during an 8-hr exposure. Thus, rate of uptake, at first rapid, diminishes with continuing exposure to a constant rate at steady state. Retention values of 60 to 80 percent have been reported at the end of short exposures (4 to 6 hours), and lower retention values would be expected with longer exposures (Fernandez et al., 1976; Monster et al., 1979; Bolanowska and Golacka, 1972). These values indicate pulmonary uptake of PCE is rather high. Since the difference in retention, 20 to 40 percent, represents excretion by other than pulmonary routes (principally metabolism), these retention values suggest that metabolism of PCE may be considerably greater than indicated by other measures of metabolism (Section 5.2.2).

Monster et al. (1979) have subjected volunteers to controlled inhalation exposures for 4 hours and determined experimentally PCE uptake kinetics. Monster et al. found that net pulmonary uptake decreased with exposure duration, with about 25 percent less PCE absorbed in the fourth hour as compared with the first hour of exposure. Table 5-2 gives the amounts (mg) PCE uptake at 72 and 144 ppm (488 and 976 mg/m^3) exposure for 4 hours. For 6 subjects, the average uptake was 455 mg and 945 mg for the two exposure concentrations, respectively, showing a direct proportionality of uptake with inspired air concentration at least up to 144 ppm (976 mg/m^3). Their data (Table 5-2) also demonstrate the marked increase in uptake rate (and total uptake) as a result of an increase in ventilation rate provoked by exercise. Interperson variations in PCE uptake were also influenced by total body mass and adipose tissue mass.

Fernandez and co-workers (Gubaran and Fernandez, 1974; Fernandez et al., 1979) and Stewart and co-workers (Hake and Stewart, 1977; Stewart et al., 1961, 1970, 1974) have also conducted controlled exposures with human volunteers. Their results are in accord with those of Monster and co-investigators. Hake and Stewart (1977) exposed five subjects for 5 consecutive days to 100 ppm (678 mg/m^3) PCE for 7 hours daily. As shown in Figure 5-5, the concentration of PCE in exhaled breath of the 5 subjects increased as the 5-day week progressed, indicating an increase in the body burden with repeated daily exposure.

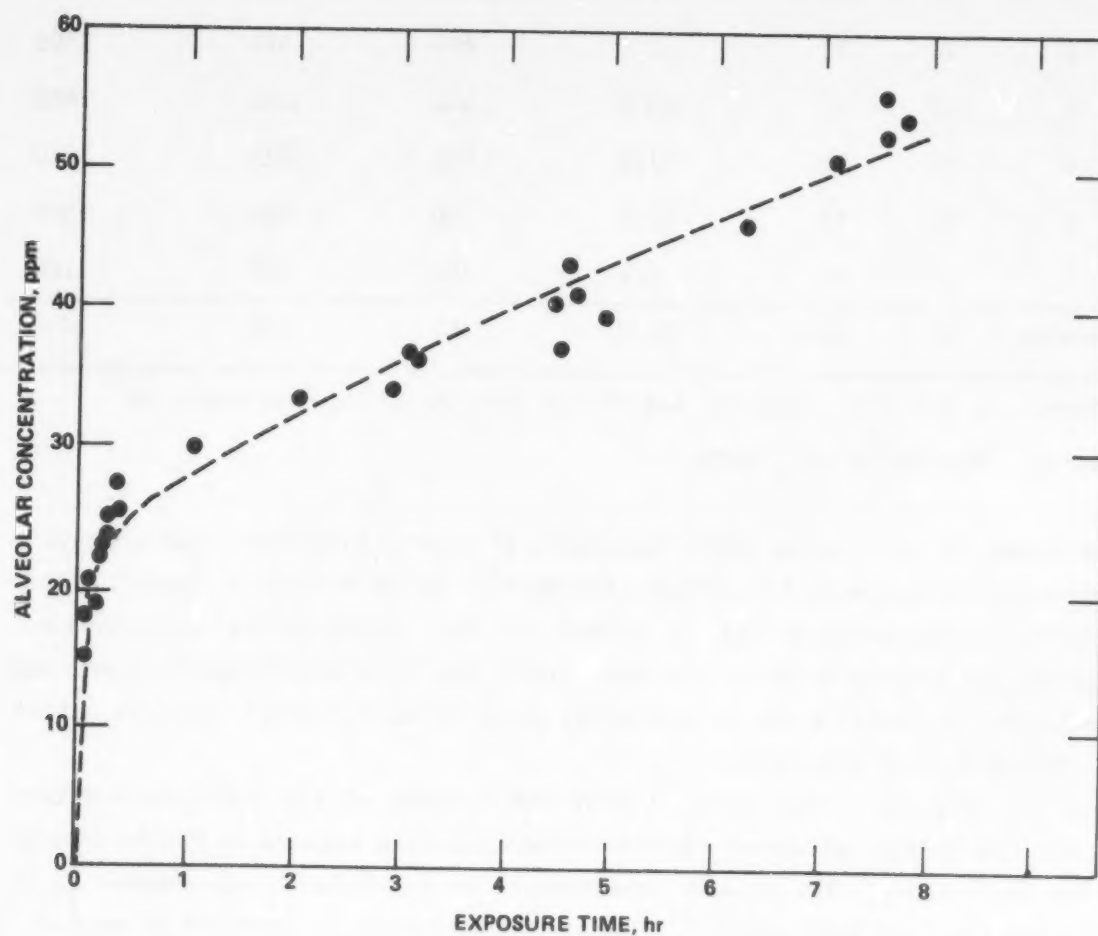


Figure 5-4. PCE alveolar air concentration during exposure of 5 subjects for 8 hr at 100 ppm. From Fernandez et al., 1976.

TABLE 5-2. ESTIMATED UPTAKE OF SIX INDIVIDUALS EXPOSED TO PCE AT REST AND AFTER EXERCISE

Subject	Body Mass (kg)	Lean body mass (kg)	Ventilation Min volume at rest (L/m)	72 ppm (at rest) (mg)	Uptake* 144 ppm (at rest) (mg)	144 ppm + exercise (mg)
A	70	62	7.6	370	670	1060
B	82	71	11.6	490	940	1500
C	82	71	10.0	530	1000	1400
D	86	74	11.3	500	1210	1510
E	67	61	12.3	390	880	1320
F	77	61	8.8	450	970	1120
Average	77.3	66.7	10.3	455	945	1318

*Exposures for 4 hr; exercise two 30-min periods during 4-hr exposure.

Source: Monster et al., 1979.

Fernandez et al., using their mathematical model, predicted that chronic occupational exposure to 100 ppm (678 mg/m³) PCE (8 hr/day, 5 day/wk) would result in cumulation of PCE in adipose tissue. As uptake by fatty tissue during the working hours of the week equals the elimination during nights and weekends, an equilibrium is eventually established but which requires a time period of 3 to 4 work weeks.

5.1.3.2 Rodents. Pegg et al. (1979) and Schumann et al. (1980) have determined the overall pulmonary uptake of rats and mice exposed to PCE by inhalation for 6 hours. The animals were exposed in a 30-liter glass chamber to 10 or 600 ppm (67.8 or 4068 mg/m³) ¹⁴C-PCE. At termination of exposure (6 hours), the cumulative pulmonary uptake was estimated by determining the radioactivity of carcass, and radioactivity in expired air, urine, and feces. The data for rats and mice are given in Tables 5-3 and 5-4, respectively. For mice, the body burden resulting from a 10-ppm exposure was 0.40 mg/animal (16.5 mg/kg), and for rats, 1.48 and 77.5 mg/animal (5.9 and 310 mg/kg) for 10 and 600 ppm (67.8 or 4068 mg/m³), respectively. These data indicate that pulmonary uptake

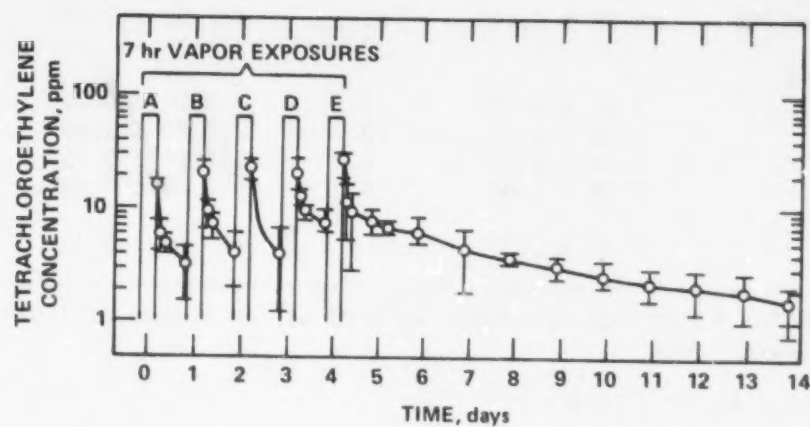


Figure 5-5. Mean exhaled breath concentrations of PCE for five volunteers exposed to 100 ppm PCE for 7 hr per day for 5 days. Note the rising concentration of PCE toward equilibrium with inspired air concentration with daily exposure, and the long decay of PCE concentration in expired air after the last exposure. (From Hake and Stewart, 1977; Stewart et al., 1970).

TABLE 5-3. DISPOSITION IN RATS OF ^{14}C -PCE RADIOACTIVITY 72 HR FOLLOWING ORAL OR INHALATION ADMINISTRATION

	Recovery, $\mu\text{mol equ. PCE}$			
	1 mg/kg*	%	500 mg/kg	%
Expired air				
Unchanged PCE	1.05 $\pm$ 0.00	71.5	667.31 $\pm$ 22.72	89.9
Metabolized				
$^{14}\text{CO}_2$	0.04 $\pm$ 0.01	2.5	3.44 $\pm$ 0.36	0.5
Urine	0.24 $\pm$ 0.00	16.5	34.48 $\pm$ 2.40	4.6
Feces	0.09 $\pm$ 0.02	6.2	29.08 $\pm$ 4.01	3.9
Carcass	0.05 $\pm$ 0.00	3.3	8.50 $\pm$ 0.66	1.2
Total	1.46 $\pm$ 0.02		742.79 $\pm$ 27.75	
	<u>10 ppm, 6 hr*</u>		<u>600 ppm, 6 hr</u>	
Expired air				
Unchanged PCE	6.08 $\pm$ 0.44	68.1	412.38 $\pm$ 7.60	88.0
Metabolized				
$^{14}\text{CO}_2$	0.32 $\pm$ 0.05	3.6	3.25 $\pm$ 0.17	0.7
Urine	1.66 $\pm$ 0.12	18.7	27.40 $\pm$ 1.68	6.0
Feces	0.46 $\pm$ 0.02	5.2	14.24 $\pm$ 0.75	3.1
Carcass	0.38 $\pm$ 0.06	4.3	10.07 $\pm$ 0.54	2.2
Total	8.91 $\pm$ 0.33		467.05 $\pm$ 8.11	

*Mean $\pm$ SE for 3 rats; gavage doses in corn oil in volume of 1.0 mL/kg; Sprague-Dawley rats.

Source: Pegg et al., 1979.

of PCE by the rat at least is approximately proportional to exposure concentration.

On a comparative species basis, the pulmonary uptake (comparing 10 ppm, 6-hr exposures) for the rat and mouse more closely relates to the ratio of their surface areas ($\text{b.w. rat}^{2/3}/\text{b.w. mouse}^{2/3} = 4.7$) than to their relative body weights (ratio 10.2). Recently, Landry et al. (1983) have developed methods to measure simultaneously respiratory frequency, tidal volume, minute volume, and net uptake of an inhaled vapor in rats. During steady state, if metabolism is the only significant route of elimination, then net uptake rate of the inhaled vapor is equal to the rate of metabolism. This new approach

TABLE 5-4. DISPOSITION IN MICE OF ^{14}C -PCE RADIOACTIVITY 72 HR FOLLOWING ORAL OR INHALATION ADMINISTRATION

	Recovery, μmol equ. PCE			
	10 ppm, 6 hr. *	%	500 mg/kg	%
Expired air				
Unchanged PCE	0.29 ± 0.06	12.0	53.69 ± 4.96	82.6
Metabolized				
$^{14}\text{CO}_2$	0.19 ± 0.01	7.9	0.85 ± 0.67	1.3
Urine	1.53 ± 0.20	62.5	6.58 ± 3.19	10.3
Feces	0.16 ± 0.04	6.8	0.80 ± 0.05	1.2
Carcass	0.07 ± 0.01	3.0	0.32 ± 0.07	0.5
Cagewash	0.19 ± 0.11	7.7	2.67 ± 1.53	4.1
Total	2.44 ± 0.27		64.92 ± 3.09	

*Mean $\pm$ SD for 3 rats; gavage doses in corn oil; $\text{B}_6\text{C}_3\text{F}_1$ mice.

Source: Schumann et al., 1980.

appears to be useful for inhalation dosimetry and evaluation of metabolic rates in rats.

5.1.4 Tissue Distribution and Concentrations

PCE is expected to distribute, by first-order diffusion processes, into all body tissues, as is known for other chloroethylene compounds with lower lipid solubility (Table 5-1; Waters et al., 1977). PCE readily crosses the blood-brain barrier and the placental barrier. The partition coefficients for various tissues, relative to blood or air, have not been firmly established, although approximations can be calculated from the rat tissue distribution data of Savolainen et al. (1977) (Table 5-5); rat tissue/blood: adipose, 60; liver, 5; brain, 4. Absolute tissue concentrations are directly proportional to the body burden or exposure dose.

5.1.4.1 Rodents. Savolainen et al. (1977) analyzed tissue levels of PCE after exposure of rats 6 hours daily for 5 days to 200 ppm (1356 mg/m^3) PCE in air. Values are shown in Table 5-5. Seventeen hours after exposure on day four, blood and other tissues still contained significant concentrations of PCE with highest concentrations in adipose tissue ($103 \mu\text{g/g}$) reflecting the high solubility of PCE in this tissue and its long storage or half-time of

TABLE 5-5. RAT ORGAN CONTENT OF PCE AFTER DAILY INHALATION EXPOSURE OF 200 PPM FOR 6 HOURS PER DAY. MEASUREMENTS MADE ON FIFTH DAY.

Time (hr of exposure on fifth day)	Cerebellum	Cerebrum	Lungs	Liver	Perirenal Fat	Blood
nmol/g (mean of two determinations $\pm$ range)						
0 (17 hr after day 4 exposure)	18.4 ± 3.7	13.1 ± 4.4	9.5 ± 1.8	35.2 ± 9.3	622.2 ± 20.0	4.3 ± 1.1
2	89.7 ± 16.7	62.0 ± 7.1	45.6 ± 10.1	107.4 ± 27.3	977.6 ± 174.0	21.3 ± 4.0
3	108.7 ± 4.9	72.0 ± 4.5	52.4 ± 9.8	134.9 ± 1.0	807.1 ± 38.0	25.3 ± 1.5
4	101.5 ± 17.2	68.1 ± 5.5	59.5 ± 13.4	133.8 ± 0.7	1105.3 ± 109.7	24.4 ± 3.4
6	142.5 ± 0.2	92.3 ± 1.3	74.0 ± 3.6	160.7 ± 24.2	1724.8 ± 422.4	30.3 ± 6.6

Source: Savolainen et al., 1977.

desaturation. With exposure on day five, tissue cumulation occurred in brain, lungs, liver, fat, and blood, and steady-state level was not achieved by the end of the 6-hr exposure.

5.1.4.2 Man. Except for blood concentrations (Figure 5-1), no direct analytical information of tissue levels after various exposure concentrations of PCE is available. As for the rat, tissue concentrations are expected to be proportional to exposure concentrations and to duration of exposure. Guberan and Fernandez (1974) developed a mathematical model of PCE uptake, distribution, and excretion (based on experimental data). Their physiological model predicted the distribution of PCE in the three major physiological compartmental tissue groups--vessel-rich group (VRG), muscle group (MG), and adipose tissue (FG)--during and after an 8-hr inhalation exposure of 100 ppm. Figure 5-2 shows that by the end of exposure, 50 percent of the solvent taken up by the body has been distributed to the fatty tissue, due to its high tissue-blood partition coefficient. The distribution of PCE to the other tissue groups is

related to their volume and partition coefficient and therefore is higher for the MG than for the VRG and the vessel-poor group (VPG). After exposure, the depletion of these three groups of tissues is almost complete after about 20 hours, and the continuing elimination in alveolar air is then related to the slow release of PCE from adipose tissue. Beyond about 20 hours of elimination, the rate of discharge of PCE from the adipose tissue, and therefore from the whole body, is an exponential function of time with a predicted half-life of about 71 hours. Because of this long residence of PCE in adipose tissue, repeated daily exposure (6 hr/day, 100 ppm) results in an accumulated concentration, as PCE from new exposures adds to residual concentration from previous exposures, until steady state is reached. Adipose tissue levels of PCE reach a steady-state "plateau" (dependent on inhalation concentration and daily exposure duration) and thereafter remain relatively constant. For a 6-hr day, 100 ppm (678 mg/m^3) exposure, Gubaran and Fernandez (1974) predicted that plateau concentrations in adipose tissue occur about 22 to 29 days after initial daily exposure.

5.2 EXCRETION

The total elimination of an absorbed body burden of PCE involves two major processes--pulmonary excretion of unchanged PCE and metabolism to urinary metabolites. The metabolism of PCE appears to be very limited in man and animals and is reviewed extensively in Section 5.4. PCE or its metabolites have not been reported to be excreted by other routes than lung and urine in any significant amounts. Bolanowski and Golacka (1972) have reported that the rate of PCE elimination through the skin in man is about $125 \text{ } \mu\text{g/hr}$ during a 6-hr exposure to 390 ppm (694 mg/m^3), or about 0.02 percent of the uptake/hr. Thus, pulmonary elimination is the major route of PCE excretion.

In rodents, the principal metabolites of PCE are trichloroacetic acid (TCA) and oxalic acid. In man, TCA is the predominant metabolite although oxalic acid may be unrecognized.

5.2.1 Pulmonary Elimination in Man

Pulmonary elimination of unchanged PCE after exposure occurs as a first-order diffusion process across the lungs from blood to alveolar air, depicted in a manner as an inverted reproduction of its uptake as illustrated in

Figure 5-3. From controlled experimental exposures in humans, Stewart and co-workers (Stewart et al., 1961, 1970, 1974; Hake and Stewart, 1977) have followed the body desaturation of PCE after exposure by serial breath analysis of alveolar air. Figure 5-5 shows the results following exposure of 5 subjects for 5 consecutive days to 100 ppm (678 mg/m³) PCE for 7 hours daily. At least two first-order phases of pulmonary elimination of PCE are apparent: an initial fast phase, followed by a slow predominant excretion phase with a half-time of about 65 hours. Monster et al. (1979) have also studied the kinetics of pulmonary elimination of PCE in experimental human exposures and determined from the concentration curves of PCE in exhaled air and blood (Figure 5-2) that the compound was eliminated from the body through the lungs at three different first-order rate constants with corresponding half-lives of 12 to 16 hours, 30 to 40 hours, and 55 to 65 hours, in accordance with the desaturation of three major body compartments, represented by VRG, MG, and FG compartments, respectively (Monster et al., 1979; Guberan and Fernandez, 1974). The long half-time (55 to 65 hours) of elimination of PCE from adipose tissue (FG), which is due to the high adipose tissue/blood partition coefficient of PCE and the low rate of blood perfusion of the tissue (Eger, 1963), is independent of the body burden of PCE as shown by the parallel blood and exhaled air concentration decay curves of Figure 5-2 for exposure concentrations of 72 and 144 ppm (488 and 976 mg/m³).

However, the exhaled air (or end alveolar air) concentrations and blood concentrations of PCE after exposure and throughout desaturation are proportional to the acquired body burden or exposure concentration and duration, and serve as a means for estimating body burdens, as illustrated in Figures 5-2 and 5-6 (see Section 5-3 for discussion). Guberan and Fernandez (1974) have developed a physiological kinetic model for PCE excretion in man, based on experimental findings from controlled exposures of volunteers. The alveolar concentrations during and after exposure in the excretory phase were predicted for use in estimating mean exposure in occupational and environmental situations. The predicted half-life of PCE stores in adipose tissue was 71.5 hours, in good agreement with the experimental observations of Stewart et al. and of Monster et al. The long half-time of pulmonary excretion of PCE shows that a long period is necessary to completely eliminate PCE, i.e., above five times the half-life or about 2 weeks.

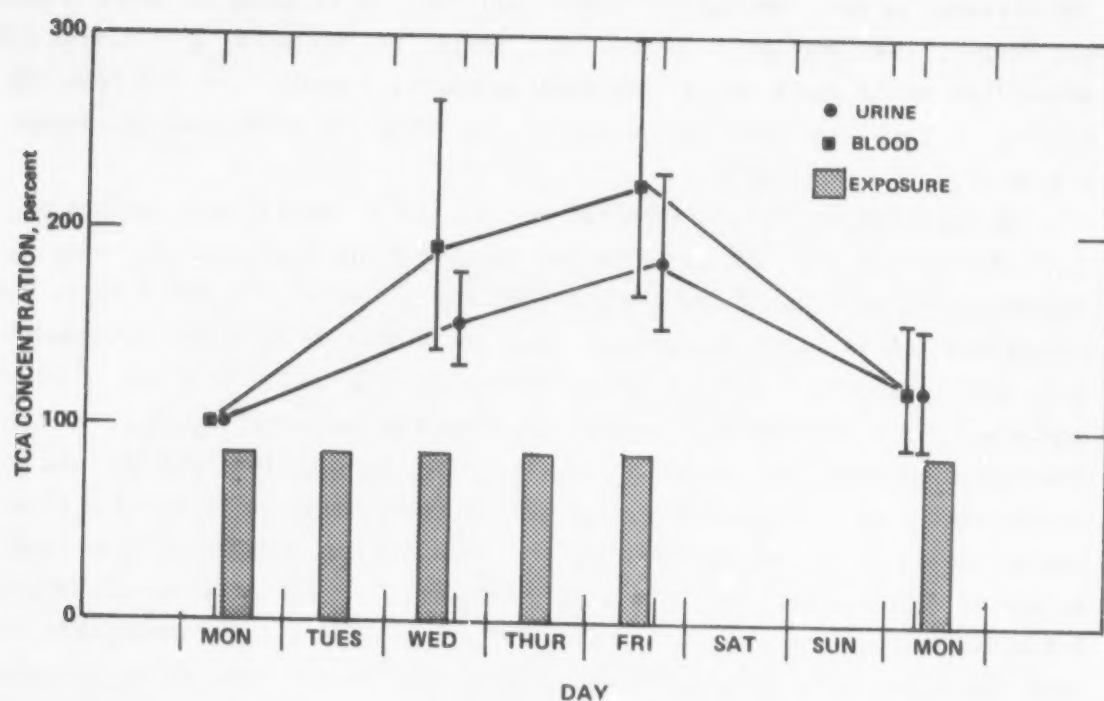


Figure 5-6. Daily (8-hr) occupational inhalation exposure to PCE. Course of the relative (first Monday morning concentration taken as 100 percent) concentration of trichloroacetic acid (TCA) in blood and urine (mean of 23 subjects $\pm$ S.D.) during the work week. From Monster et al., 1983.

5.2.2 Urinary Metabolite Excretion in Man

In contrast to first-order pulmonary excretion of PCE, the urinary excretion of TCA (or total trichloro-compounds by Fujiwara reaction) from metabolism of PCE is dose-dependent, and presumably follows Michaelis-Menten kinetics [See Section 5.4.3 for discussion]. Most studies have quantitated PCE urinary metabolite excretion by the Fujiwara reaction for which there is a question of the true nature of the compound(s) measured, although it is accepted that TCA is the principal compound. Thus Ikeda and Imamura (1973) found the mean $T_{1/2}$ of total urinary trichloro-compounds for 13 subjects occupationally exposed to PCE to be 144 hours (range 123 to 190 hours). In contrast, when TCA is directly administered to men, the half-life has been found to be 51 to 82 hours (Paykoc and Powell, 1945; Muller et al., 1974). Hence, the longer $T_{1/2}$ of TCA from PCE metabolism is no doubt due to constant metabolic formation of TCA from PCE cycling to the liver over the period of its long residence time in adipose tissue ($T_{1/2}$, 17 to 55 hours).

Monster and co-workers (Monster et al., 1979; Monster and Houtkooper, 1979; Monster et al., 1983), using gas chromatographic methods for analysis, exposed volunteers to 72 and 144 ppm (488 and 976 mg/m³) PCE for 4 hours and determined TCA in blood and urine. They found that urinary TCA represented less than 1 percent of the estimated absorbed dose of PCE. In blood, following exposure, TCA continued to increase in concentration until about 20 hours (representing continued metabolism of PCE in the body to TCA) and then declined exponentially as a first-order elimination process (Figure 5-7). The blood concentration of TCA was proportional to exposure concentration (i.e., body burden of PCE) and declined with a half-life of about 65 to 90 hours. Figure 5-8 shows that urinary cumulation paralleled blood TCA disappearance, and also that the urinary TCA cumulation was proportional to the inhalation exposure concentration of PCE (and hence body burden). Physical activity increases PCE pulmonary uptake (body burden), and hence, increased TCA formation and urinary excretion. However, more recently Ohtsuki et al. (1983) correlated inhalation exposure concentration with urinary total trichloro-compounds and found that urinary metabolite excretion was not a linear function of exposure concentration but instead a hyperbolic function approaching a "plateau" at about 400 ppm exposure (Figure 5-9). Since urinary metabolite(s) excretion reflects PCE metabolism, these observations indicate that PCE metabolism is dose-dependent and saturable with high PCE body burdens or exposure.

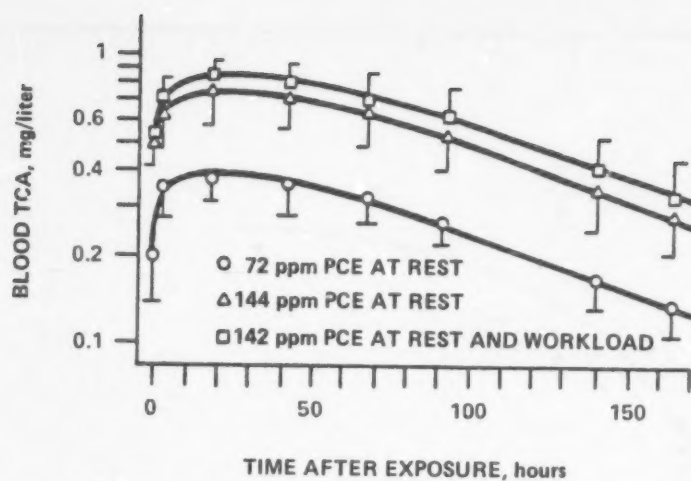


Figure 5-7. Trichloroacetic acid blood concentrations following inhalation exposure to PCE for 4 hr (means of 6 subjects). From Monster et al., 1979.

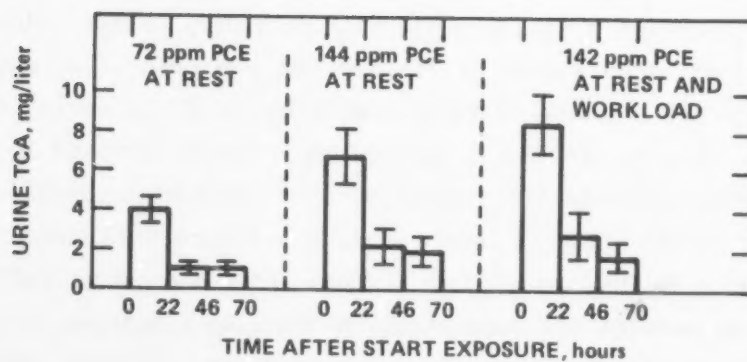


Figure 5-8. Urinary excretion of trichloroacetic acid during and following inhalation exposure to PCE for 4 hr (means of 6 subjects). From Monster et al., 1979.

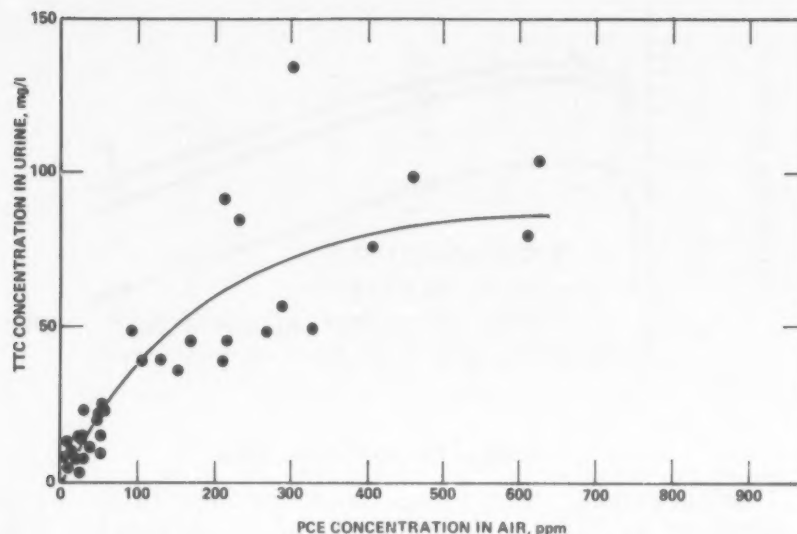


Figure 5-9. Relationship between PCE occupational inhalation exposure (time-weighted ppm average for an 8-hr work shift) and urinary concentration of total trichloro-compounds at end of work shift (corrected to a urine specific gravity of 1.016) for 36 male (squares) and 25 female workers (circles). From Ohtsuji et al., 1983.

5.2.3 Chronic Exposure

The long half-time of elimination of PCE by the pulmonary route (~ 65 hr) and by urinary metabolite(s) (~ 65 to 144 hr) indicates that, with repetitive daily or chronic inhalation exposure to PCE, sustained or "plateau" levels of PCE and TCA in blood and of TCA in urine would occur as body burden, metabolism, and excretion approach a steady state with pulmonary uptake. Monster et al. (1983) determined blood levels of PCE and TCA and urine TCA concentrations during occupational exposure (8 to 50 ppm, 8 hours daily) during the course of the work week. Figures 5-6 and 5-10 show their data expressed in terms relative to Monday morning concentrations (control; 100 percent) occurring from previous work exposure for 23 male and female workers. Figure 5-10 shows that exhaled air concentration (a measure of body burden) and blood concentration (directly proportional to exhaled air concentration and also a measure of body burden) both rose 4-fold with the 3-day exposures of Monday, Tuesday, and Wednesday, and tended to "plateau" with Thursday and Friday exposures. These "plateau" levels declined to Monday morning levels (100 percent) during exposure-free days, Saturday and Sunday. Figure 5-6 shows that the concentrations of the metabolite TCA increased during the work week in blood as well as in urine. The relative concentrations on Wednesday and Friday after work were higher by a factor of two than on Monday morning. The increase of the concentration in

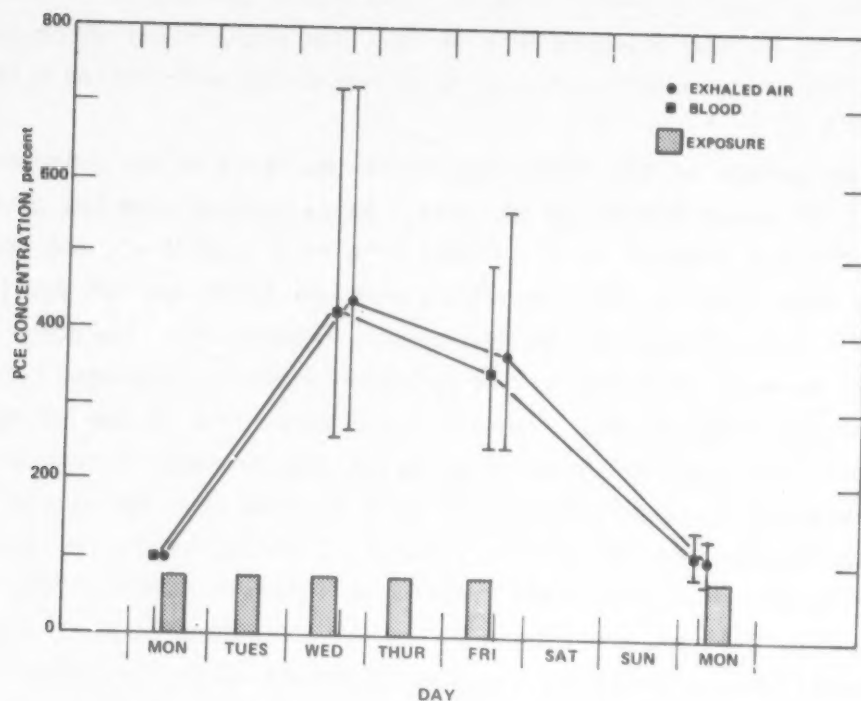


Figure 5-10. Daily (8-hr) occupational inhalation exposure to PCE. Course of the relative concentration (first Monday morning concentration taken as 100 percent) of PCE in blood and exhaled air during the work week (mean of 23 subjects $\pm$ S.D.). From Monster et al., 1983.

urine was more pronounced than that in blood. At the start of the following week the relative concentrations had returned to about 100 percent. Thus, during daily exposure during the work week, the body burden and blood levels of PCE increased with each exposure and then tended to plateau towards the end of the week, but the metabolite TCA continued to accumulate in blood and urine apparently as a result of the longer half-life of TCA. Similar experimental observations have been reported by Tada and Nakaaki (1969), and predicted from the mathematical model of Gubaran and Fernandez (1974).

5.2.4 Excretion Kinetics in the Rodent

As is the case for man, PCE is excreted from the rodent (rat and mouse) principally by two routes: pulmonary elimination of unchanged PCE and by metabolism and renal elimination of PCE metabolites. For the rat, Pegg et al. (1979) and Frantz and Watanabe (1983) found that the rate of PCE excretion in expired air is described by apparent first-order kinetics. No significant difference was observed in the elimination half-time (approximately 7 hours) with

either dose or route of administration (inhalation, gavage, or drinking water). Figure 5-1 shows the disappearance of PCE from whole blood which follows apparent first-order kinetics for up to 36 hours with elimination rate constant of 0.10 to 0.12 hr⁻¹.

The percentage of the total body burden excreted by the pulmonary route in the rat and mouse depends on the dose. As calculated from the data of Pegg et al. (1979) and Schumann et al. (1980) (Tables 5-3 and 5-4), the body burdens of PCE in rats after a 6-hr inhalation exposure to 10 and 600 ppm (68 and 4068 mg/m³) are 1.48 and 77.5 mg per animal, respectively; the percentages of these body burdens excreted by the pulmonary route as unchanged PCE were 68 and 99 percent, respectively. For the mouse exposed to 10 ppm (68 mg/m³) PCE for 6 hours, the body burden was 0.40 mg and the pulmonary excretion only 12 percent, whereas for a body burden of 10.8 mg from oral administration, 83 percent was excreted by the pulmonary route. Similar results can be calculated for body burdens from gavage and drinking water administration from the data of Tables 5-3, 5-4, and 5-6 (Pegg et al., 1979; Schumann et al., 1980; Frantz and Watanabe, 1983). Hence, as the body burden of PCE is increased in the rat or mouse, the percentage excreted unchanged increases. Conversely, as metabolism is the other principal route of elimination of PCE, when the body burden increases, the percentage of the burden metabolized (urinary metabolites) decreases (although the absolute amount increases) (Tables 5-3, 5-4, and 5-6). These observations suggest that metabolism of PCE and urinary excretion of metabolites in the rodent are rate-limited and dose-dependent, following Michaelis-Menten kinetics (whereas pulmonary excretion is a first-order process and dose-independent, i.e., with half-time and rate constant independent of dose).

Other investigators have found evidence of dose-dependent Michaelis-Menten metabolism kinetics. Filser and Bolt (1979) exposed Wistar rats to PCE in air in a closed system and determined the pharmacokinetics from disappearance rate of PCE from the chamber (uptake by rat). In comparison to other halogenated ethylenes, they found PCE to be metabolized extremely slowly in rats. Zero order V_{max} (saturation) was <1.16 mg/hr/kg b.w.; for a 250-g rat, about 3.0 mg PCE/hr is metabolized and excreted as urinary TCA and other metabolites. The principal urinary metabolites in the rat are oxalic acid and TCA (Table 5-7). Buben and O'Flaherty (1984), using Swiss-Cox mice, found urinary metabolite(s) (TCA only in these mice) excretion was limited by metabolic capacity. Mice were chronically administered PCE by gavage at dose levels up to 2000 mg/kg/day.

TABLE 5-6. DISPOSITION IN RATS OF ^{14}C -PCE RADIOACTIVITY 72 HR FOLLOWING DRINKING WATER INGESTION*

	Recovery, μmol equ. PCE	
	8.09 mg/kg*	%
Expired air		
Unchanged PCE	12.47 $\pm$ 5.15**	87.9
Metabolized		
$^{14}\text{CO}_2$	0.29 $\pm$ 0.08	2.2
Urine	0.96 $\pm$ 0.26	7.2
Feces	0.23 $\pm$ 0.07	1.7
Carcass	0.15 $\pm$ 0.04	0.9
Cagewash	0.02 $\pm$ 0.02	0.2
Total	14.10 $\pm$ 5.51	

*Rats consumed an average dose of 8.09 ± 3.13 mg/kg, which varied with the volume of drinking water ingested over a 12-hr exposure period.

**Each value represents mean $\pm$ SD from four Sprague-Dawley rats.

Source: Frantz and Watanabe, 1983.

A plot of amount of urinary metabolite (TCA) per day versus dose level fitted the Michaelis-Menten function showing the metabolism of PCE is capacity-limited even at relatively low doses (below 100-200 mg/kg) (Figure 5-11), with an estimated V_{max} for urinary metabolite excretion of 136 mg/kg/day (5.7 mg/hr/kg b.w.) and a K_m of 660 mg/kg. These values suggest an excretion by metabolism in mice greater than that estimated in the rat by Filser and Bolt (1979) by other experimental means.

5.3 MEASURES OF EXPOSURE AND BODY BURDEN

The pharmacokinetic parameters of PCE uptake, metabolism, and pulmonary excretion obtained from experimental human and animal exposures have been used to determine reliable estimates of body burdens occasioned by occupational and environmental exposures (Stewart et al., 1961, 1970, 1974; Boettner and Muranko, 1969; Ikeda et al., 1972; Essing et al., 1973; Ikeda and Imamura, 1973; Guberan and Fernandez, 1974; Fernandez et al., 1976; May, 1976; Monster et al., 1979; Monster and Houtkooper, 1979; Tada and Nakaaki, 1969; Ogata et al., 1971;

TABLE 5-7. REPORTED METABOLITES OF PCE (OTHER THAN TCA)

Metabolite	System	Investigator
Inorganic chloride	Rat urine	Daniel, 1963
Carbon dioxide	Rat, mouse exhaled air	Pegg et al., 1979 Schumann et al., 1980
Oxalic acid	Rat, mouse urine	Yllner, 1961 Pegg et al., 1979 Dmitrieva, 1967
Dichloroacetic acid	Mouse urine	Yllner, 1961
Ethylene glycol	Rat urine	Dmitrieva, 1967
Production from PCE-oxide Spontaneous Decomposition in Aqueous and Non-aqueous Solution		
Trichloroacetic acid		Bonse et al., 1975
Trichloroacetyl chloride		Kline et al., 1978

Verberk and Scheffers, 1980; Ziglio, 1981; Ohtsuki et al., 1983; Monster et al., 1983; Monster and Smolders, 1984). From these studies, three approaches have been developed to estimate body burdens of PCE: 1) concentration of PCE in exhaled air, 2) blood levels of PCE, and 3) concentrations of trichloroacetic acid (TCA) in blood and urine. Stewart et al. (1961) pioneered the use of expired air concentrations as a measure of body burden. In addition to the advantage of a non-invasive methodology, alveolar PCE concentrations appear to be a good index of the vapor exposure to which individuals have most recently been subjected (Figure 5-12). Urine analysis for TCA excretion appears to be of lesser value in estimating exposure to PCE. The urinary excretion of TCA increases slowly and gains the maximal level only after 1 to 4 days, and furthermore, the level is not clearly proportional to inhalation concentration (Weiss, 1969; Tada and Nakaaki, 1969; Kundeg and Hogger, 1970; Ikeda et al., 1972; Fernandez et al., 1976; Ohtsui et al., 1983), owing to the long half-life of PCE metabolites that appear in urine (144 hr, Ikeda, 1977) and to a low metabolic rate which is dose-dependent and saturable (Section 5.4.3). Monster and co-workers (Monster et al., 1983; Monster and Smolders, 1984) find

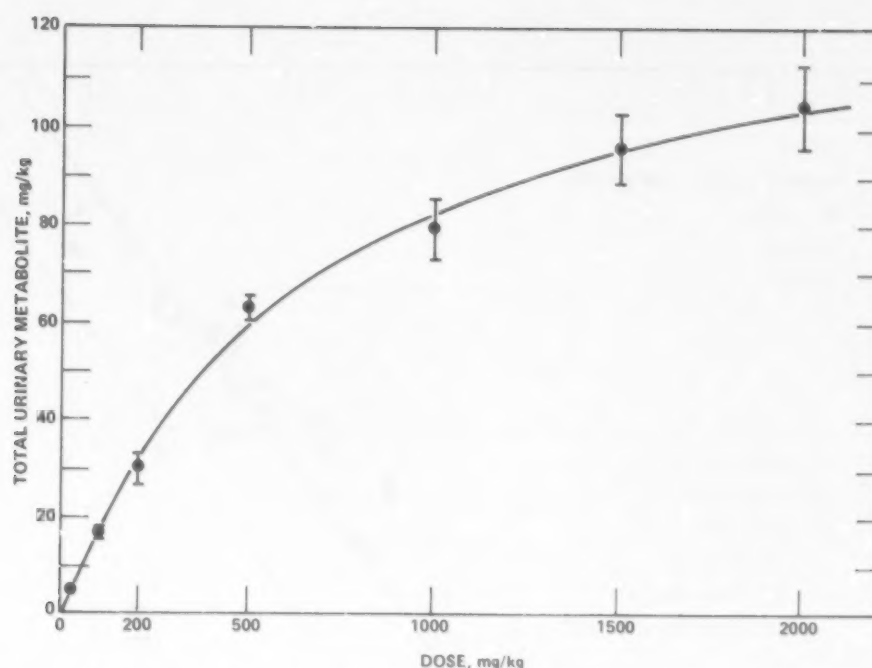


Figure 5-11. Relationship between PCE and dose and the amount of total urinary metabolite excreted per day by mice. Data points are means of 9 to 11 mice $\pm$ SEM. The points fit the Michaelis-Menten equation with an r^2 value of 0.996. The calculated value from the curvilinear fit are $V_{max} = 136$ mg/kg/day and $K_m = 660$ mg/kg. From Buben and O'Flaherty, 1984.

that for measuring environmental exposure to PCE, biological monitoring of exhaled air is a "simple, efficient, effective and convenient method" of assessing total ambient exposure of both young and aged subjects. For measuring occupational exposure, they find that the best parameter to estimate the time-weighted average exposure to PCE over the whole work week is the exhaled concentration of PCE and TCA in blood at the end of the workday on Friday. The second best parameter is measurement of blood PCE and exhaled air concentration.

These methods for estimating PCE body burdens after single or chronic exposure are subject to high inter-individual variations that limit their predictive value. Some of the factors known to contribute to these variabilities are pulmonary dysfunctional states, individual differences in intrinsic metabolic capacity for PCE, body mass and adipose tissue mass, modification of metabolism by drugs and environmental xenobiotics, age, sex, and exercise or workload. Guberan and Fernandez (1974), using a mathematical model developed to predict uptake and distribution of PCE in the body and its elimination in alveolar air, have computer-simulated the effects of age, body weight, height, and body fat content, both at rest and during physical exercise.

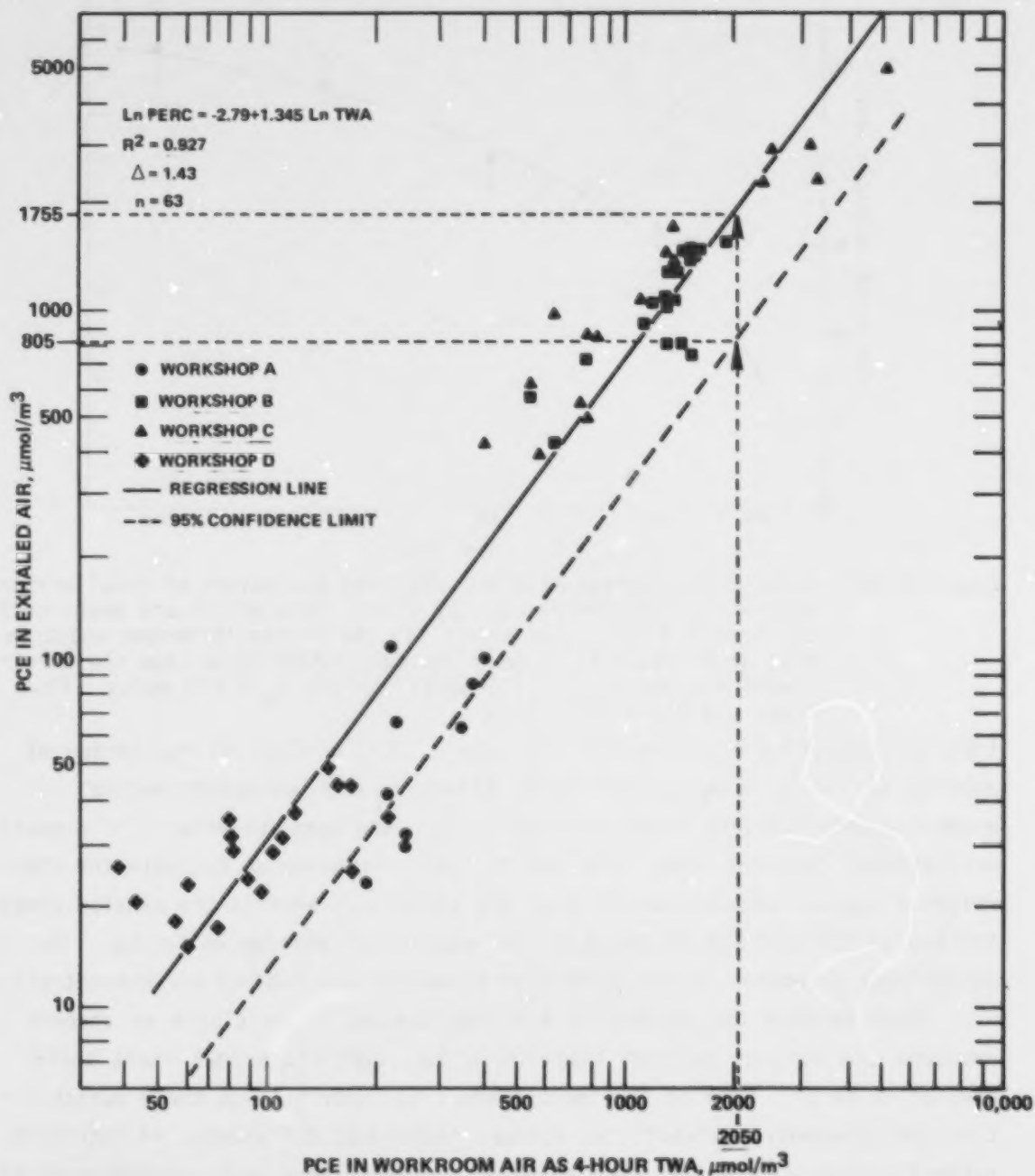


Figure 5-12. Direct linear relationship between the time-weighted average occupational exposure to PCE over the last 4 hr of a work day and the concentration of PCE in exhaled air 15-30 min after the end of exposure (data for 32 subjects). Inhalation exposure concentration of 2,050 $\mu\text{mol}/\text{m}^3$ is equivalent to 50 ppm. From Monster et al., 1983.

5.4 METABOLISM

5.4.1 Known Metabolites

Trichloroacetic acid (TCA) is the most prominent metabolite of PCE in animals and man. The principal site of metabolism is the liver. Bonse et al. (1975) have perfused the isolated rat liver with PCE and directly demonstrated the capacity of this organ to metabolize PCE to TCA; however, no other metabolite (e.g., oxalic acid) was found. Other tissues, such as lung and kidney, may also metabolize PCE, as these tissues are known also to contain P-450 metabolizing systems. Nonetheless, other metabolites have also been described (Table 5-7). The metabolism of PCE was first systematically investigated with ^{14}C -labelled compound some 25 years ago by Yllner (1961). Yllner established the principal urinary metabolites of mice to be TCA (52 percent), oxalic acid (11 percent) with traces of dichloroacetic acid, and the remaining radioactivity (37 percent) in unknown form. More recently, Dmitrieva (1967) and Pegg et al. (1979) have confirmed that oxalic acid is a major urinary metabolite of rats, and, indeed, in this species, may be the principal metabolite. As yet, oxalic acid has not been determined to be a metabolite of man, for whom TCA is presently assumed to be the principal metabolite. Buben and O'Flaherty (1984) observed no excess oxalic acid excreted by mice after chronic administration of large oral doses of PCE, and these workers suggest that oxalic acid is not a significant metabolite in this species.

The identification of trichloroethanol (TCE) in the urine of humans has been described by some authors (Ogata et al., 1962; Tanaka and Ikeda, 1968; Ikeda and Ohtsuji, 1972; Ikeda et al., 1972). However, in all of these studies, the method used for TCA metabolite quantitation (Fujiwara reaction) is based on color production before and after oxidation with chromium trioxide and addition of pyridine. The difference between the color production before (TCA) and after oxidation is considered an estimate of TCE content. Sakamoto (1974), from comparative urine analysis by GC and the Fujiwara reaction under different pH and temperature conditions, expresses doubt that the entire fraction detected by Fujiwara reaction in the oxidation fraction is truly TCE. Nonetheless, Monster et al. (1983) and also Weicherd and Lindner (1975) identified by GC small amounts of TCE ($<4 \mu\text{mol}/\text{mmol}$ creatinine) in the urine of persons exposed to 10 to 30 ppm (68 and $204 \text{ mg}/\text{m}^3$) PCE in air, although others using GC have not in controlled experimental exposures to pure PCE (Hake and Stewart, 1977; Fernandez et al., 1976; Monster et al., 1979). For mice, Yllner

(1961) reported that by his chromatographic method TCE was not detected in urine as a PCE metabolite. Daniel (1963) reported similarly for the rat, using steam distillation combined with isotopic dilution methodology.

Buben and O'Flaherty (1984) also were unable to find evidence of TCE in the urine of chronically dosed mice as analyzed by gas chromatography; TCA was the only metabolite found. Furthermore, Costa and Ivanetich (1980) could not detect TCE as a product of PCE metabolism by pre-induced rat liver microsomal preparations in vitro.

In mammalian systems, oxalic acid is known to be converted in part to carbon dioxide, which has been identified in exhaled air of the rat and mouse after exposure to ^{14}C -labelled PCE (Table 5-4). Dmitrieva (1967) has also reported substantial amounts of ethylene glycol in urine of rats exposed to PCE, although this observation has not been confirmed by others (Buben and O'Flaherty, 1984). As no complete balance study of end-metabolites of PCE has been reported for the rodent or man, it is likely that all metabolites of PCE have not been identified in these species. At present, there is insufficient evidence for assuming a qualitative species difference in PCE metabolism.

5.4.2 Enzymic Pathways of Metabolism

The pathways of PCE metabolism are speculative. The currently accepted pathway for the production of TCA from PCE is shown in Figure 5-13. This pathway was initially proposed by Powell in 1945 for trichloroethylene and was subsequently supported for PCE by the results of Yllner (1961), Daniel (1963), Liebman and Ortiz (1970, 1977), Costa and Ivanetich (1980) and others. The first step in this pathway appears to be catalyzed by hepatic cytochrome P-450 system to give 1,1,2,2-tetrachloroethylene oxide, although formation of this epoxide has not been rigorously verified by chemical isolation or identification as an intermediate to TCA formation in microsomal reactions. However, the evidence is strong as reviewed below. The epoxide has been chemically synthesized (Frankel et al., 1957; Bonse et al., 1975; Kline et al., 1978). In sodium phosphate buffer at pH 7.4, the oxide hydrolyzes at a pseudo-first order rate of $6 \times 10^{-2} \text{ min}^{-1}$ ($T_{1/2}$ -12 min) to TCA, and thermally decomposes in nonaqueous solution to trichloroacetyl chloride (Table 5-4). Sakamoto (1976) also synthesized the epoxide in milligram amounts, essentially by the method of Frankel et al. (1957), and determined urinary metabolites after i.p.

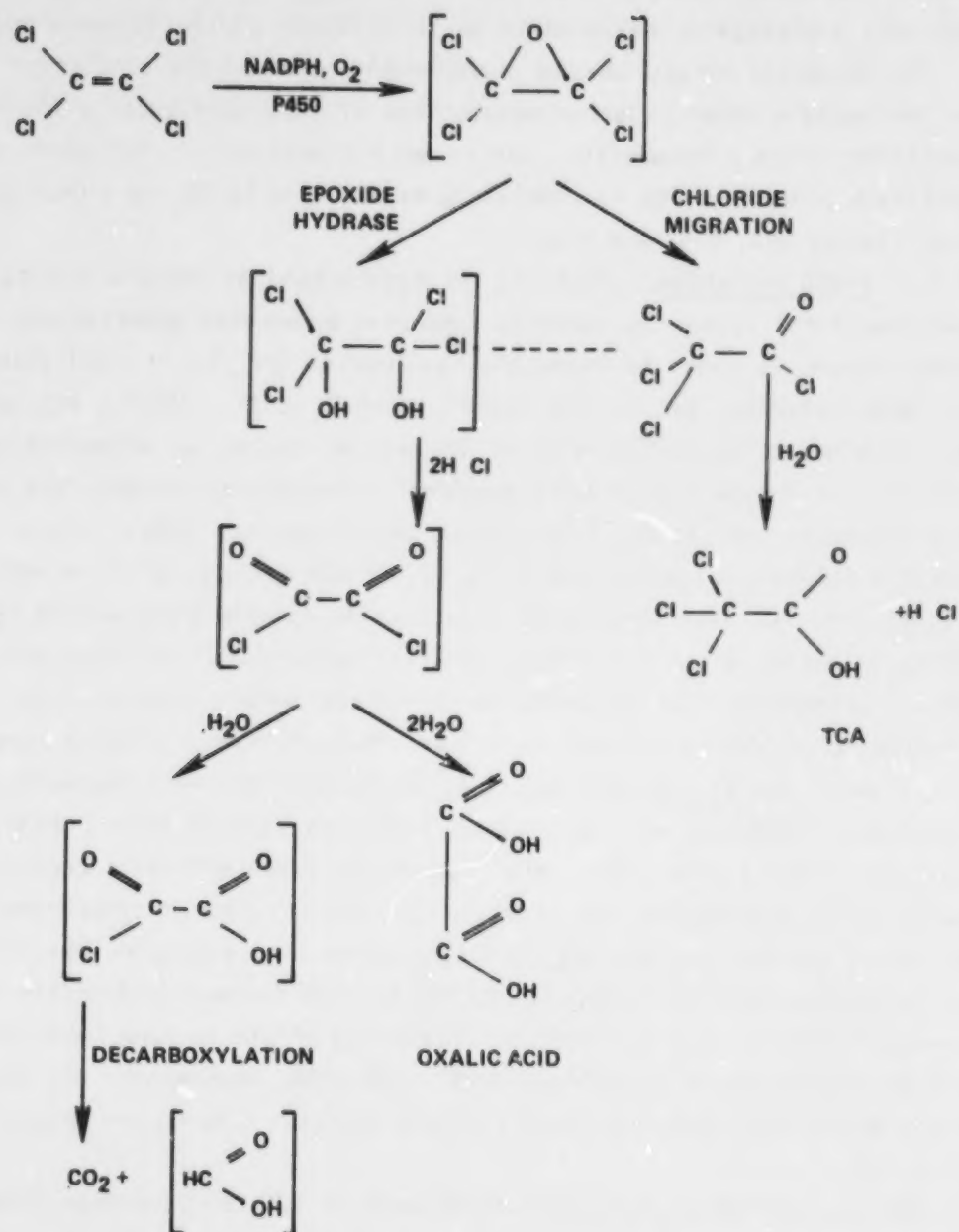


Figure 5-13. Postulated scheme for the metabolism of PCE. (After Pegg et al., 1979; Costa and Ivanetich, 1980).

injection into guinea pigs. By GC analysis, TCA was the only metabolite; the Fujiwara reaction, however, indicated the presence of a small amount of TCE which this investigator believed to be an artifact of the Fujiwara method.

The enzymatic or spontaneous nonenzymatic steps in PCE conversion to TCA does not explain other reported metabolites of PCE, particularly the well-established urinary metabolite, oxalic acid (Table 5-7). Furthermore, a significant portion of PCE is completely metabolized to CO_2 in a dose-dependent manner (Tables 5-3, 5-4, and 5-6).

5.4.2.1 P-450 oxidation. That PCE is metabolized by hepatic microsomal cytochrome P-450 system is strongly supported by several observations. This enzymic system is known to metabolize analogs of PCE, i.e., vinyl chloride, vinylidene chloride, and trichloroethylene (Bolt et al., 1982). PCE has been shown to bind to the active site of the enzyme system, as evidenced by the production of a Type I difference spectrum in hepatic microsomes from rats in vitro (Pelkonen and Vaino, 1975; Costa and Ivanetich, 1980). Costa and Ivanetich (1980) and Liebman and Ortiz (1977) demonstrated with rat microsomes in vitro that PCE also stimulated nicotinamide adenine dinucleotide reduced (NADPH) oxidation which is characteristic of cytochrome P-450-dependent reactions. Furthermore, TCA was produced during the aerobic incubation of hepatic microsomes (O_2 ; NADPH required) in a time-dependent manner (Figure 5-14). The K_M (1.1 mmol) and $V_{\max}$ (0.046 nmol TCA/min/nmol P-450) were respectively decreased and increased with microsomes from rats treated with inducers of cytochrome P-450 (Table 5-8). With respect to phenobarbital induction, the data of Table 5-8 suggest the induction of one iso-form of cytochrome P-450 that has a greater PCE binding equilibrium and also a greater reactivity, i.e., a greater ability to metabolize PCE by both increasing 2.5-fold hepatic microsomal content of P-450 and also increasing 4-fold average P-450 reactivity. Finally, inhibitors of cytochrome P-450 (SKF 525A, metyrapone, CO) added to hepatic microsomes with PCE inhibited both spectral binding and metabolism of PCE to TCA.

The equilibrium constant for the binding of PCE to cytochrome P-450 ($K_S = 0.43 \text{ mmol}$) is similar to that of trichloroethylene (0.4 mmol). However, the maximum rates of uninduced metabolism ($V_{\max}$) of PCE by the cytochrome P-450 system are approximately 30-fold lower than the corresponding rate for the metabolism of trichloroethylene (0.046 versus 1.55 nmol TCA/min/nmol P-450) (Table 5-8; Costa et al., 1980).

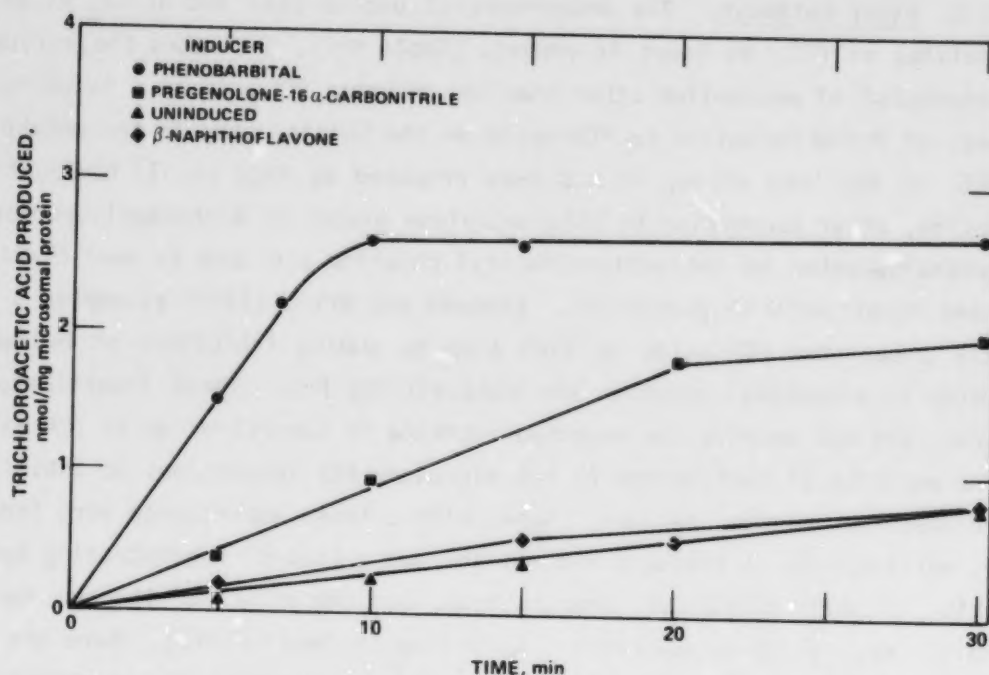


Figure 5-14. Production of TCA from PCE by hepatic microsomes from different-ly pretreated rats as a function of time. Incubation mixtures contained in 0.2 M Tris-HCl (pH 7.4), tetrachloroethylene (3.3 mM), NADPH-generating systems, EDTA (0.2 mM), and hepatic microsomes (2 mg protein/ml) from uninduced (triangle), β -naphthoflavone-induced (diamond), pregnenolone-16 α -carbonitrile (square) and phenobarbital-induced (circle) male Long-Evans rats. (From Costa et al., 1980).

TABLE 5-8. METABOLISM OF PCE BY RAT HEPATIC MICROSOMES AND THE EFFECT OF VARIOUS INDUCERS

Inducing Agent	P-450 content nmoles/mg microsomal protein	K_M (mM)	V_{max} nmoles TCA/min/ nmole P-450
None	$0.89 \pm 0.06^*$	1.1 ± 0.8	0.046 ± 0.004
β -naphthoflavone	1.03 ± 0.01	0.5 ± 0.5	0.055 ± 0.014
Pregnenolone-16 α carbonitrile	1.50 ± 0.11	2.4 ± 0.8	0.16 ± 0.09
Phenobarbital	2.32 ± 0.32	0.2 ± 0.1	0.19 ± 0.02

*Mean $\pm$ S.D.; measured in presence of 3.3 mM PCE.

Source: Costa et al., 1980.

5.4.2.2 Other Pathways. The occurrence of oxalic acid and of CO_2 as major metabolites of PCE, at least in rodents (Table 5-7), indicates the existence of pathway(s) of metabolism other than the primary TCA pathway. Assuming the primacy of P-450 oxidation to PCE-oxide as the initial step in the metabolism of PCE, as outlined above, it has been proposed by Pegg et al. (1979) that PCE-oxide, after conversion to chloroethylene glycol by microsomal hydrase, is dehydrohalogenated to tetrachlorodiacetyl chloride and then to oxalic acid or CO_2 and formic acid (Figure 5-13). Liebman and Ortiz (1977) attempted a test of the postulated PCE-oxide to diol step by adding inhibitors of hydrase activity to microsomal preparations metabolizing PCE. These investigators, however, did not observe the expected increase in the PCE-oxide to TCA pathway by the addition of cyclohexene to rat microsome-PCE incubations to inhibit the postulated epoxide-diol pathway. Nonetheless, these experiments were inconclusive, particularly as the data did not include a control demonstrating hydrase activity in their microsomal preparations, and the effect of the cyclohexene inhibitor with a known substrate. According to Oesch (1972), there are two types of epoxide hydrase, one of which is tightly coupled to the cytochrome P-450 system and one of which is not; the coupled form is thought to be more important in detoxifying epoxide formed by mixed-function oxidases and is relatively resistant to inhibitors.

The postulated pathways of PCE metabolism (Figure 5-13) do not include conjugation reactions as possible metabolic detoxification steps. Presently, there is little evidence that PCE-oxide might conjugate with glutathione (GSH), for example, as a detoxification pathway as postulated for vinylidene chloride-oxide (Jones and Hathway, 1978; Reichert et al., 1979). Pegg et al. (1979) found that inhalation exposure of rats to 600 ppm (4068 mg/m^3) PCE for 6 hours had no significant effect on total hepatic nonprotein sulfhydryl, an estimate of hepatic GSH content. GSH concentration was estimated as 5.06 ± 0.02 and $4.93 \pm 0.21 \text{ } \mu\text{mol/mg}$ liver for control and exposed animals, respectively. The pathways for the origin of major metabolites of PCE other than TCA (Table 5-7) are deserving of further attention and investigation.

5.4.3 Magnitude and Dose-Dependency of Metabolism

Experimental human and animal exposures to PCE have been carried out with an overall finding that PCE metabolism is very limited, i.e., that saturation of mammalian capacity to PCE occurs at relatively low levels of exposure.

5.4.3.1 Man. Estimates of the extent of metabolism in man, as a percentage of retained PCE, have been made from balance studies by accounting for a retained dose after inhalation exposure by measuring metabolites (TCA, total trichloro-compounds by Fujiwara reaction) excreted in the urine (Stewart et al., 1961, 1970; Monster et al., 1979; Monster and Houtkooper, 1970; Monster et al., 1983; Boettner and Muranko, 1969; Ikeda et al., 1972; Essing et al., 1973; Fernandez et al., 1979; May, 1976). Only about 1 to 3 percent of the estimated amounts absorbed are metabolized into TCA and other chlorinated metabolites. Ikeda and co-workers (Ikeda et al., 1972; Ohtsuki et al., 1983) have explored the relationship of inhalation exposure concentration in occupational settings (time-weighted 8-hr average) metabolite concentration in urine (total trichloro-compounds; Fujiwara reaction) at the end of an 8-hr work shift. Under these conditions, these investigators found a saturation of metabolism occurs between 100 to 400 ppm (678 to 2112 mg/m³) PCE in inspired air (Figure 5-9). They estimated that at the end of an 8-hr shift with exposure to PCE at 50 ppm (339 mg/m³) (below saturation; Figure 5-9), 38 percent of PCE pulmonary uptake would be exhaled unchanged, and less than 2 percent would be metabolized to urinary chlorinated compounds, while the rest would remain in the body to be eliminated in succeeding hours.

The difficulties associated with balance studies in man are the problems encountered with (1) accurate measurement of the retained dose of PCE from inhalation exposure, (2) the imprecision of the older methodologies using the Fujiwara reaction for metabolite quantification, (3) the possibilities that metabolites other than TCA (e.g., oxalic acid, TCE-glucuronide) may be excreted in urine or by biliary excretion, and (4) the very long half-life (144 hours) of PCE urinary metabolites which necessitates extended collection of samples. For example, Bolanowski and Golacka (1972) in their series of experimental exposures to PCE in man found that the elimination of TCA in the urine represents only a few percent of the dose while the alveolar retention of PCE was 60 to 80 percent (Fernandez et al., 1976; Monster et al., 1979). This suggests a total metabolism of 20 to 40 percent of the dose. Thus they assume that another unrecognized pathway exists for PCE which has not yet been taken into consideration. Conversion of PCE to CO₂ could constitute a significant portion of this metabolism.

5.4.3.2 Animals. For rodents (rat and mouse), the extent of metabolism after single doses of PCE has been estimated by balance studies using isotopically labelled PCE. Yllner (1961) carried out the earliest study in mice. By

exposing the mice by inhalation to ^{14}C -PCE vapor, he found that of the radioactive PCE absorbed (2 hr-exposure; 0.9 mg/kg b.w.), 70 percent was excreted unchanged in expired air, 2 percent was metabolized and excreted in the urine, and 0.5 percent in the feces over a four day post-dosage period. Daniel (1963) gavaged rats with ^{36}Cl -PCE (about 1.12 and 8.29 mg/g b.w.) and found that 98 percent of these doses were excreted unchanged by the lungs and only 2 percent of radioactivity was metabolized and excreted in the urine over a 2 to 18-day period (Table 5-9). These early observations, which indicate a very limited metabolism of PCE by rodents, are in accord with the more recent kinetic studies of Filser and Bolt (1979). These investigators exposed rats to PCE in a closed system and measured the rate of disappearance of PCE vapor. The kinetics of metabolism of PCE was dose-dependent and the estimated V_{max} was less than 1.2 mg/kg/hr. Filser and Bolt noted that PCE was metabolized extremely slowly in rats (in comparison to other halogenated ethylenes) and they were unable to experimentally differentiate between zero order and first order kinetics and hence to determine the saturation point, i.e., ppm PCE in air at which metabolism "saturation" occurs.

Other investigators have also found evidence of dose-dependent Michaelis-Menten kinetics for metabolism of PCE in mice and rats following both oral and inhalation exposure. Pegg et al. (1979) exposed rats to single oral doses of ^{14}C -PCE (1 and 50 mg/kg b.w.) and to single inhalation exposures (10 and 600 ppm for 6 hours) and followed the disposition of radioactivity in exhaled air, urine, and feces for 3 days post administration. Their data are given in Table 5-3. For these low doses (1 mg/kg and 10 ppm, 6 hours), 71.5 and 68.1 percent, respectively, of the body burdens were excreted as unchanged PCE in exhaled air. The remainder, 28.5 and 31.9 percent, was metabolized to CO_2 and fecal and urinary metabolites. In contrast, for the higher oral and inhalation exposure doses (500 mg/kg and 600 ppm, 6 hours), a greater percentage of the body burdens was exhaled as unchanged PCE (89.9 and 88.0 percent, respectively). Hence, these data demonstrate that even at very low body burdens of PCE in the rat (approximately 0.25 to 2.5 mg per animal) only 30 percent is metabolized, and this percentage decreased with increasing body burden. This indicates that metabolic disposition of PCE is a saturable, dose-dependent process in this species after either oral (gavage) or inhalation exposures. Recently, Frantz and Watanabe (1983) have demonstrated that PCE from saturated solutions of drinking water, allowed ad libitum over a 12-hr period to rats, provided

TABLE 5-9. DISPOSITION OF ^{36}Cl -PCE AFTER ORAL ADMINISTRATION TO WISTAR RATS

Dose (μC)***	Mg/Kg Equivalents	No. Animals	Expired air* (unchanged) PCE	Metabolized	
				Urine**	Feces
1.75	1116	1	97.9	2.1	0
13	6160	4	--	1.6	0

*Collected for 48 hr.

**Collected for 18 days.

***Specific activity of ^{36}Cl -PCE was 1.3 $\mu\text{C}/\text{mmol}$.

Source: Daniel, 1963.

average body burdens of 8.09 ± 3.13 mg/kg (2.3 mg per animal), and gave comparable results to low dose (1 mg/kg) gavage dosage, i.e., 88 percent of the body burden of PCE was excreted unchanged in exhaled air and only approximately 12 percent was metabolized. Their results are shown in Table 5-6.

Schumann et al. (1980) have investigated the extent of metabolism in mice using ^{14}C -labelled PCE. The experimental conditions were very similar to those of Pegg et al. (1979) and Frantz and Watanabe (1983) in rats as these experiments originated from the same laboratory. Schumann and co-workers determined the disposition in mice of body burdens of PCE of 0.04 mg per mouse (from 10 ppm, 6 hours inhalation exposure) and of 10.8 mg per mouse (from 500 mg/kg gavage dose) (Table 5-4). For the smaller body burden, 12 percent was excreted unchanged in exhaled air and the remainder (about 88 percent) metabolized; for the larger body burden, the proportions were reversed with 83 percent of the body burden excreted unchanged and about 17 percent disposed of by metabolism. These data indicate that for mice also, metabolism of PCE is dose-dependent and saturable for this species. When compared to rats (Tables 5-3 and 5-6 versus Table 5-4), mice were found to metabolize 8.5 and 1.6 times more PCE per kg body weight following inhalation of 10 ppm ($68 \text{ mg}/\text{m}^3$) for 6 hours or a single oral dose of 500 mg/kg respectively. However, on a body burden per animal basis, the rat metabolized 6.8 and 1.3 times more PCE than the mouse following inhalation of 10 ppm ($68 \text{ mg}/\text{m}^3$) for 6 hours or a single oral

dose of 500 mg/kg respectively. Thus, from these observations, the metabolism of PCE by these two species is more consistent with a metabolism proportional to body surface area than to kg body weight.

Buben and O'Flaherty (1984) have also investigated the extent of PCE metabolism in male mice (Swiss-Cox strain) using urinary metabolite excretion as an index of metabolism. These investigators daily dosed the mice by gavage with PCE in corn oil, 5 days per week for 6 weeks, at dose levels of 20, 100, 200, 500, 1000, 1500, and 2000 mg/kg/day. TCA was the only metabolite found in the urine of this strain with less than 5 percent of the dose excreted in the feces. Nonetheless, total urinary metabolite as an index of metabolism is only a dose approximation since it is known that some metabolized compound is bound to cellular macromolecules and some is metabolized to CO_2 (Tables 5-3, 5-4, and 5-6). However, urinary excretion is by far the major route of disposition of metabolized PCE. Therefore, total urinary metabolite should be a reasonable approximation of the amount of metabolism. Also, with chronic administration, body burden of PCE is expected to reach steady-state, and in fact these investigators found no week-to-week trends in daily urinary metabolite output although urinary TCA tended to increase during the week with plateauing towards the end of the week; urine collections were made at the weekly plateau. Figure 5-12 shows the relationship found between the amount of metabolism and the administered dose. The data clearly show a dose-dependent metabolism consistent with capacity limited kinetics in accordance with Michaelis-Menten equation, $Y = \frac{M_{\max} \cdot X}{K_m + X}$, where X is the dose (mg/kg) and Y is mg/day of metabolite resulting. From the computer-fitted equation, Buben and O'Flaherty estimated $M_{\max}$ (maximum rate of metabolite formed and excreted in 24 hr) to be 136 mg/kg/day and K_m (the dose at which the amount of metabolite excreted in a 24-hr period is half the apparent maximum amount) at 660 mg PCE/kg. These workers also estimated that at a very low chronic oral dose of PCE only 25 percent was metabolized (indicating significant hepatic flow-limited or first pass effect) and less than 5 percent of a very high dose. These observations also indicate that, in this strain of mice at least, the metabolism of PCE deviates from linearity (i.e., from first order) at oral doses above 100 to 200 mg/kg. Of considerable importance also is the finding of a clear relationship between toxicity and metabolism of PCE. Figure 5-15 shows a direct linear relationship between hepatotoxicity (serum SGPT activity) and urinary metabolite excretion. Similar relationships were observed with

other indices of hepatotoxicity such as liver weight increase, liver triglyceride accumulation, and glucose-6-phosphatase activity. These observations are consistent with the formation of a toxic or reactive intermediate(s) in the primary metabolic pathway of PCE (Figure 5-13).

5.4.4 Covalent Binding

PCE is known to bind to the substrate binding site of hepatic microsomal cytochrome P-450 in vitro (Costa et al., 1980). A direct relation also has been demonstrated among the level of hepatic microsomal cytochrome P-450, the extent of metabolism of PCE in vivo, and cellular damage (Bonse et al., 1975; Moslen et al., 1977; Pegg et al., 1979; Schumann et al., 1980; Buben and O'Flaherty, 1984). For example, Moslen et al. (1977), in a study designed to correlate metabolism with hepatocellular toxicity, observed that induction of microsomal systems in rats by phenobarbital or chlorinated biphenyls increased metabolism of orally administered PCE (5.7-fold) and increased indices of toxicity (2-fold). Buben and O'Flaherty (1984) have also presented evidence for a strong correlation between hepatocellular toxicity and metabolism. As shown in Figure 5-15, these workers found in mice linear relationships between the amount of metabolism of PCE (as measured by urinary metabolites) and hepatotoxicity as measured by several indices of toxicity. In terms of the proposed metabolic pathway(s) for PCE (Figure 5-13), covalent binding to cellular macromolecules, leading to cellular damage, may occur with putative reactive intermediates of PCE metabolism: PCE-oxide, trichloroacetyl chloride, and chlorodiacyl chloride. Bonse et al. (1975) and Bolt et al. (1982) have proposed that the symmetrical PCE oxide metabolite is much less reactive than the epoxides of unsymmetrical chlorinated ethylenes such as trichloroethylene, vinylidene chloride, and vinyl chloride. Consequently, covalent binding of PCE metabolites to cellular macromolecules may result in less severe hepatic toxicity and genotoxicity, even when taking into account the large differences in the hepatic abilities to metabolize these compounds (Section 5.4.3). This correlation between biological activity and chemical stability has been confirmed by Jonas and Mackrodt (1982), who studied the relationship between the mutagenic activity of chlorinated ethylenes and the C-O bond energy of their epoxides.

Bonse et al. (1975) perfused isolated rat liver preparations with PCE (180 ppm concentration) and observed that only 3 to 5 percent of the total uptake bound to liver tissue; this portion was extractable only after acid

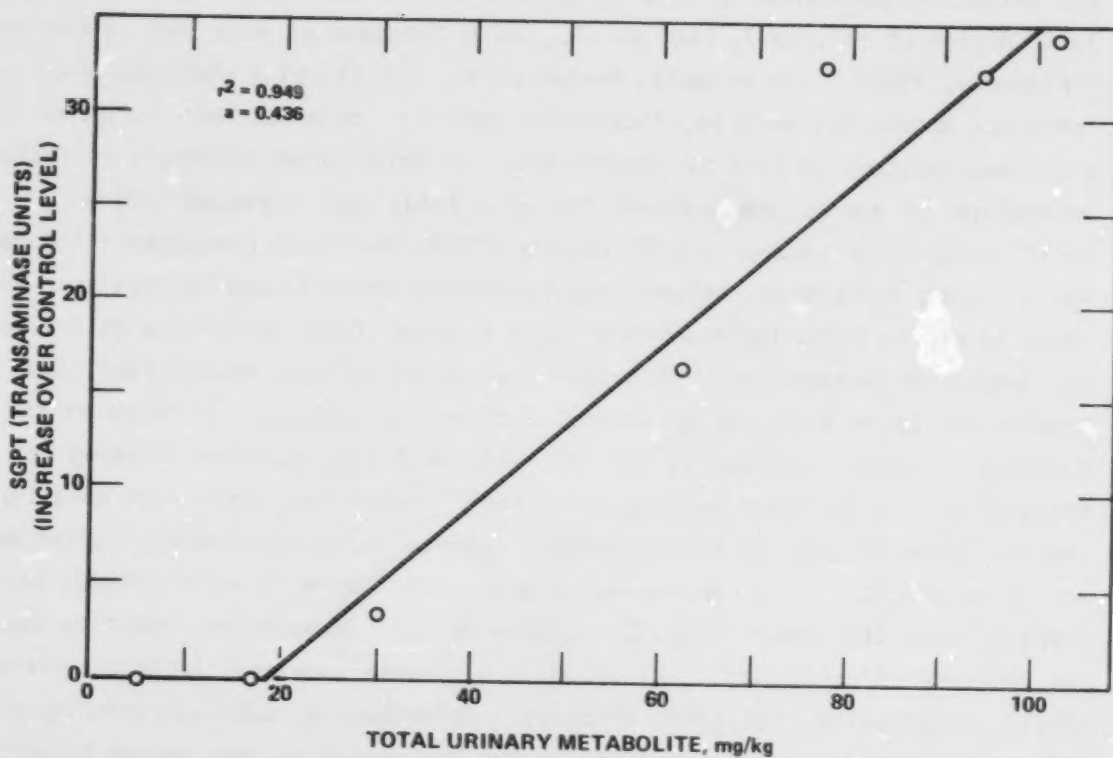


Figure 5-15. Relationship between hepatotoxicity parameters from PCE oral administration (shown as serum SGPT activity increases) and total urinary metabolite excreted per day by mice at increasing dose levels of PCE. The slope of the linear regression line is 0.436 with a coefficient of regression, r^2 , of 0.949. From Buben and O'Flaherty, 1984.

hydrolysis. They proposed that the acyl chloride intermediate in the conversion to TCA (10 to 15 percent of uptake) reacted with cell functional groups, e.g., -OH, -SH, and -NH₂. Pegg et al. (1979) have demonstrated *in vivo* rat hepatic binding after exposure to PCE by both inhalation and oral routes. Their data are shown in Table 5-10. Radioactivity irreversibly associated with tissue macromolecules was assayed 72 hr after termination of inhalation exposure to 10 or 200 ppm (68 or 1356 mg/m³) for 6 hr and 72 hr after oral administration of ¹⁴C-PCE. Irreversible binding (primarily to cellular protein and nucleic acid) was dose-dependent and metabolism-dependent, but independent of the route of exposure. The ratio of amount bound to total amount metabolized (about 1 percent; Table 5-10) was not significantly different between high and low doses of PCE by either route of exposure, suggesting that irreversible binding was solely a function of metabolism and the availability of reactive species. The persistence of irreversible bound metabolites of PCE (72 hr after exposure) indicates that turnover rates of the bound metabolite(s) are slow enough that accumulation could potentially occur in man with repeated daily exposures.

TABLE 5-10. IRREVERSIBLE HEPATIC BINDING OF ¹⁴C-PCE IN RATS
72 HR AFTER EXPOSURE

Route	Exposure	Amount Metabolized μmol eq	Bound μmol eq/ liver protein	Bound/ Metabolized x 100
Oral	1 mg/kg	0.41 ± 0.03*	0.0035 ± 0.0003	0.83
	500 mg/kg	73.92 ± 4.98	0.4321 ± 0.0477	0.59
Inhalation (6 hr)	10 ppm	2.82 ± 0.16	0.0245 ± 0.0022	0.87
	600 ppm	55.46 ± 0.44	0.4029 ± 0.0222	0.72

*Mean ± S.E.

Source: Pegg et al., 1979.

Schumann et al. (1980) have compared metabolism of PCE and irreversible hepatic binding in the two species, rat (Sprague-Dawley) and mouse (B₆C₃F₁). Their data are summarized in Table 5-11. The time course of hepatic macromolecular binding differs between these two species; the peak binding occurs in mice significantly more rapidly than in rats after exposure from both

TABLE 5-11. COMPARISON OF IRREVERSIBLE HEPATIC BINDING OF ^{14}C -PCE IN SPRAGUE-DAWLEY RATS AND $\text{B}_6\text{C}_3\text{F}_1$ MICE AFTER INHALATION (6 HR) AND ORAL EXPOSURES

Time, hr post- exposure	$\mu\text{g mol-eq bound/ g hepatic protein}$					
	600 ppm			500 mg/kg		
	A. Mice	B. Rats	A/B	A. Mice	B. Rats	A/B
0	$1.604 \pm 0.251^*$	0.174 ± 0.064	9.2	-	-	-
1	-	-	-	0.964 ± 0.115	0.170 ± 0.025	5.7
6	1.355 ± 0.120	0.227 ± 0.026	6.0	2.171 ± 0.523	0.331 ± 0.027	8.2
12	-	-	-	1.687 ± 0.112	0.402 ± 0.086	4.2
24	1.027 ± 0.138	0.279 ± 0.017	3.7	1.386 ± 0.262	0.612 ± 0.027	2.3
48	0.729 ± 0.232	0.246 ± 0.062	3.0	1.081 ± 0.290	0.347 ± 0.060	3.1
72	0.481 ± 0.127	0.171 ± 0.050	2.8	0.472 ± 0.131	0.321 ± 0.060	1.5

*Mean $\pm$ S.D.

Source: Schumann et al., 1980.

inhalation and oral routes of administration. There is 3 to 5 times more binding per gram hepatic protein in mice than rats. Since liver weight of these two species is proportional to species body weight, the total hepatic-bound PCE is comparable to the metabolism of PCE by the mouse and rat (Section 5.4.3.2).

Schumann et al. (1980) also have tried to evaluate the extent of binding to DNA and its relation with the probability of genotoxicity. These workers utilized the mouse in their experiments because of greater binding per gram of liver in this species, and because of the susceptibility of this species to hepatic tumors (NCI, 1977). These investigators did not find a detectable binding of high specific activity ^{14}C -PCE (1.3×10^5 dpm/ μmol) to liver DNA when mice were exposed by inhalation (600 ppm) or by gastric intubation (500 mg/kg). However, the limit of detection was estimated as 10 to 14.5 alkylations per 10^6 nucleotides. Therefore, levels of DNA binding below their detection limit may have occurred. In parallel experiments, these investigators (Schumann et al., 1980; Watanabe et al., 1980) also chronically administered PCE (11 to 12 daily doses) by gavage at increasing dose levels up to 1000 mg/kg (Table 5-12). Mice, but not rats, showed a 20-25 percent increase in liver/body weight ratio. DNA/g liver protein decreased, indicating that

TABLE 5-12. EFFECTS OF CHRONIC ORAL ADMINISTRATION OF PCE ON HEPATIC DNA CONTENT AND DNA SYNTHESIS IN MICE AND RATS^a

Dose (mg/kg/day)	Liver DNA content (mg DNA/g liver)		Liver DNA synthesis (dpm [³ H]thymidine/μg DNA)	
	Mice	Rats	Mice	Rats
12 doses in 16 days				
0	4.00 ± 0.42	2.22 ± 0.29	65 ± 34	63 ± 11
500	3.31 ± 0.22*	2.03 ± 0.17	118 ± 22*	65 ± 17
11 doses in 11 days				
0	2.51 ± 0.11	2.33 ± 0.19	44 ± 15	88 ± 24
100	2.29 ± 0.10*	2.24 ± 0.22	56 ± 27	46 ± 26
250	2.25 ± 0.17*	2.51 ± 0.16	63 ± 26	137 ± 103
500	2.23 ± 0.15*	2.36 ± 0.24	75 ± 23	105 ± 45
1000	2.30 ± 0.10*	2.18 ± 0.27	82 ± 31*	108 ± 45

^a Mean ± SD (n = 3-7); [³H]thymidine 1000 μCi/kg i.p. 6 hr prior to sacrifice.

* p < 0.05.

Source: Schumann et al. (1980).

organ weight gain resulted from hypertrophy rather than hyperplasia. Nonetheless, DNA synthesis (¹⁴C-thymidine incorporation) tended to increase in both mice and rats in a dose-related manner, suggesting some activation of "repair" mechanisms.

5.4.5 Interactions with Metabolism

5.4.5.1 Induction and Inhibition. The metabolism of PCE is known to be increased by inducers of the cytochrome P-450 oxidative system (microsomal mixed-function monooxygenases). Moslen et al. (1977) demonstrated that the metabolism of PCE in intact rats, as measured by urinary metabolites, was enhanced (4 to 7-fold) by pretreatment with inducers of cytochrome P-450, phenobarbital and polychlorinated biphenyls, which increased the hepatic cytochrome content 2.4 and 3.3-fold, respectively. Similar results have been reported by Ikeda and Imamura (1973) for both intact rats and hamsters. Costa and Ivanetich (1980) found that pretreatment of rats with the inducers, phenobarbital and pregnenolone-16α-carbonitrile, increased cytochrome P-450 content of microsomes and markedly enhanced the metabolism of PCE to TCA by these

microsomes in vitro (Figure 5-14). Inhibitors of cytochrome P-450, SKF 525A, metyrapone, and CO, added to the microsomal reaction medium, significantly decreased the production of TCA.

Chronic exposure to PCE appears to produce minimal self-induction of metabolism. Kaemmerer et al. (1982) report that feeding rats 25 and 200 ppm PCE in their diet resulted in a small but statistically significant induction of cytochrome P-450 in rat liver homogenates after 14 and 7 days treatment, respectively. Vainio and co-workers (Savolainen et al., 1977; Vainio et al., 1976) found a slight depressing effect on rat liver P-450 content 24 hours after a single high oral dose of PCE (1700 mg/kg) but reported slight increases after PCE inhalation exposure at 600 ppm (4068 mg/m^3), 6 hours daily for 5 days. Plevova et al. (1975) and also Kaemmerer et al. (1982) observed that in rats, exposure to 177 ppm (1200 mg/m^3) PCE for 6 hours, 20 hours prior to i.p. administration of pentobarbital (44 mg/kg), lengthened by 30 percent the pentobarbital sleeping time. These observations suggest that substrate competition for cytochrome P-450 system for the metabolism of pentobarbital and for PCE may occur, with a consequent increase of rate of metabolism of phenobarbital and prolongation of its half-life.

5.4.5.2 Drugs. In addition to the potential for PCE interactions with clinical drugs, some of which are known to induce P-450 system or compete for P-450 oxidative metabolism, other types of interactions are possible. For example, Hake and Stewart (1977) evaluated the effect of diazepam and of alcohol, CNS depressants, on human exposure to PCE. During an 11-week study with inhalation exposure to PCE, 5.5 hours per day at 25 and 100 ppm (169 and 678 mg/m^3), concomitant administration of ethanol (blood levels 30 to 100 mg percent) increased PCE blood levels in the 25 ppm exposure but not with 100 ppm; diazepam (blood levels 7 to 30 μg percent) had little effect. Additionally, behavioral and neurological tests revealed no interactive effects under these conditions. However, May (1976) found that urinary excretion of TCA was completely inhibited by a blood ethanol concentration of 0.65 mg percent.

5.4.5.3 Other Interactions. PCE has been observed to have a significant effect on intermediary metabolism. Takano and Miyazaki (1982) and Miyazaki and Takano (1983) reported that the PCE inhibited glutamate, succinate, and malate oxidation by rat liver mitochondria. The lesser inhibition of succinate oxidation suggested to the authors that PCE may act as an uncoupler in the electron transport system between NADH dehydrogenase and coenzyme Q. Ogata

and Hasegawa (1981) have reported similar findings for PCE and other halocarbons for succinate oxidation by rat liver mitochondria. Since both the K_m and V_{max} of the reactions were affected by PCE, it was suggested that PCE represents a nonspecific inhibitor (possible mitochondrial membrane perturbation by this lipid solvent) rather than a specific inhibitor like cyanide or antimycin A.

5.5 SUMMARY

Perchloroethylene is rapidly and virtually completely absorbed from the gastrointestinal tract of rats and mice after administration of doses up to 500 mg/kg by intragastric intubation, and of doses up to 8 mg/kg in drinking water, with peak blood concentrations occurring within one hour of dosing. Pulmonary uptake of PCE during inhalation exposure by experimental animals and man is linearly proportional to exposure duration and concentration in air; it is also influenced by physical activity and body mass. For rats exposed to 10 and 600 ppm (68 and 4068 mg/m³) for 6 hours, net uptake of PCE has been measured as 5.9 and 310 mg/kg b.w.; for man exposed to 72 and 144 ppm (488 and 976 mg/m³) for 4 hours, the net uptake averages 6.5 and 13.5 mg/kg. Steady-state uptake in man is not established within short (8 hours) exposure periods. With chronic exposure (100 ppm; 8 hr/day; 5 days/wk), 3 to 4 weeks is required for steady state. Absorption of PCE during vapor or liquid contact with the skin of experimental animals or man is very slow and adds less than 1 percent to the body burden.

PCE distributes widely into body tissues, and readily crosses the blood-brain barrier and the placental barrier. Highest tissue concentrations are found in the adipose tissue (60 times blood level), and in brain and liver (4 and 5 times blood level, respectively). Tissue concentrations increase in direct proportion to the body burden. With chronic exposure in man, adipose tissue concentrations do not achieve plateau concentrations until 3 to 4 weeks of exposure.

PCE is eliminated from the body principally by pulmonary excretion of unchanged compound and also by a rate-limited metabolism of PCE. Pulmonary excretion in man and experimental animals occurs in three first-order phases of desaturation of blood vessel-rich tissue group (VRG), muscle tissues (MG), and adipose tissues (FG). For man, the half-times of elimination from these groups are 12-16 hr, 30-40 hr, and 55-65 hr, respectively. For rat, the dominant half-time of pulmonary elimination is about 7 hours.

The capacity of man and rodents to eliminate PCE by metabolism is limited. For man, as assessed by urinary metabolite excretion, about 1-3 percent of the absorbed amounts of PCE from exposures of 100 to 400 ppm (678 and 2112 mg/m³) exposure for 8 hours are metabolized, with a half-time of renal elimination of the metabolites of about 144 hours. Urinary metabolite excretion increases as PCE body burden increases until a plateau is reached at about 400 ppm (2112 mg/m³) exposure (500 to 600 mg body burden). Metabolism is, therefore, dose-dependent and saturable in man. Similar observations have been made for rodents. For the rat and mouse, the percentage of the administered dose that is metabolized decreases with increase of dose. Metabolism in both the rat and mouse is consistent with saturable, dose-dependent Michaelis-Menten kinetics with a maximum rate (V_{max}) in the order of 1.6 mg/hr/kg b.w. for the rat and 5.7 mg/hr/kg for the mouse.

Indices of hepatotoxicity of PCE in the rat and mouse are highly correlative with the dose-dependent nature of the metabolism of PCE in these species. The principal site of metabolism appears to be hepatic P-450 monooxygenase system where PCE is oxidized to PCE oxide, which rearranges to trichloroacetic acid (TCA). TCA has been identified as a major urinary metabolite in man, rat, and mouse. Metabolism to TCA, in vitro and in vivo, is known to be increased by inducers of the P-450 oxidative system. Secondary pathways of metabolism may include hydration of PCE oxide with subsequent dehydrohalogenation of the glycol to chlorodiacetyl chloride and further rearrangement to yield oxalic acid or formic acid and carbon dioxide. In rodents, oxalic acid is a urinary metabolite while carbon dioxide is a pulmonary metabolite. There is no evidence that conjugation reactions with glutathione or cysteine are important in the metabolism of PCE. While the metabolite profiles of PCE in man, rat, and mouse are not as yet fully established, there is no convincing evidence of qualitative or quantitative differences of pathways in these species.

PCE metabolite(s) are known to covalently bind, in vitro and in vivo, to cellular macromolecules such as protein and lipids. Tissue binding appears to be solely a function of metabolism and availability of reactive metabolites. Since tissue-bound metabolites have a slow rate of turnover and accumulate, cumulative cellular damage may occur with chronic exposure. Covalent binding to hepatic DNA in vivo is minimal; however, as the limit of detection for these experiments was only 10 to 14.5 alkylations/10⁶ nucleotides, very low levels of DNA binding cannot be completely excluded.

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6. TOXIC EFFECTS

6.1 HUMANS

The known effects of tetrachloroethylene (perchloroethylene, PCE) on humans have been established primarily from individuals accidentally or occupationally exposed to very high (in nearly all cases, unknown) concentrations of PCE. Exposure to high concentrations of PCE causes a variety of toxicological effects in humans. Effects upon the central nervous system (CNS) are generally the most noticeable following acute or excessive occupational exposures. Effects upon the liver and kidney usually are observed after an elapsed period of exposure to high concentrations.

6.1.1 Effects on the Liver

6.1.1.1 Acute Exposure Situations--Associations between liver damage and acute exposures of humans to high air levels of PCE have been reported by several investigators (Stewart et al., 1961a; Stewart, 1969; Saland, 1967; Hake and Stewart, 1977; Levine, 1981).

Clinical evidence of impaired liver function was found by Stewart et al. (1961a) in a worker rendered semi-comatose by overexposure to a petroleum-based solvent estimated to contain about 50 percent PCE. Simulation of the exposure suggested that during the 3.5-hr exposure, the last 30 minutes probably averaged about 1000 ppm (6780 mg/m^3) PCE; the average estimated concentration during the initial 3 hours was about 275 ppm (1864 mg/m^3). At such levels, metabolic saturation was exceeded. A neurological examination conducted 1 hour after the worker's collapse indicated no abnormality of function; also, the liver was not palpable. During a 6-week period subsequent to the incident, neither clinical jaundice nor neurological deficits were observed. Beginning 9 days postexposure, urinary urobilinogen and total serum bilirubin were elevated. Serum glutamic-pyruvic transaminase (SGPT) and serum glutamic-oxaloacetic transaminase (SGOT) were in the normal range throughout the postexposure period. On the 18th day, SGPT was slightly elevated. On the 16th postexposure day, the PCE level in expired air was sharply elevated. The investigators suggested that such an acute overexposure may represent a continuing insult to the liver since PCE had an exceedingly long exponential decay in expired air, indicating slow release from body tissues.

In another case report, Stewart (1969) diagnosed transient and mild hepatitis in a worker exposed to anesthetic levels of PCE for 30 minutes.

Urinary urobilinogen was elevated on the 9th postexposure day. SGOT was slightly elevated on the 3rd and 4th postexposure days. Stewart concluded this patient had experienced marked depression of the central nervous system followed by transient, minimal liver injury.

Hake and Stewart (1977) reported mild liver injury, as indicated by elevated serum enzymes, in a 60-yr-old male overcome by PCE vapors. The individual was reported to have fully recovered.

In the reports by Levine et al. (1981) and Saland (1967), a cause-effect relationship between PCE and the effects observed cannot be made.

An enlarged liver and obstructive jaundice were diagnosed by Bagnell and Ellenberger (1977) in a 6-wk-old, breast-fed infant. While this situation is not uncommon in infants, the infant had been indirectly exposed to PCE. The child's father worked as a leather and suede cleaner in a dry-cleaning establishment where PCE vapors were present. During regular lunchtime visits to the exposure site, the mother had been exposed to the same vapors. These visits lasted between 30 and 60 minutes. The concentration of PCE in the work place was unknown, although it was believed to be excessively high because of reported episodes of dizziness. In the infant, bilirubin, SGOT, and serum alkaline phosphatase were elevated; other blood and urinary parameters of liver function were normal. Normal liver function was found in both parents. Analysis of the mother's blood 2 hours after one of her lunchtime visits indicated a PCE level of 0.3 mg per 100 mL. One hour after a visit, her breast milk contained 1.0 mg PCE per 100 mL. After 24 hours, the concentration of PCE in the breast milk decreased to 0.3 mg per 100 mL. Chlorinated hydrocarbons were not found in the mother's urine. One week after breast feeding was discontinued, serum bilirubin and serum alkaline phosphatase levels in the infant returned to a normal range. The findings suggest that the neonatal liver may be sensitive to toxicological effects of PCE, although other causal factors in this instance cannot be ruled out.

6.1.1.2 Chronic Exposure Situations--Alterations in liver function in persons exposed to unknown concentrations of PCE over extended periods have been reported by a number of investigators (Coler and Rossmiller, 1953; Franke and Eggeling, 1969; Hughes, 1954; Trense and Zimmerman, 1969; Meckler and Phelps, 1966; Larsen et al., 1977; Moeschlin, 1965; Dumortier et al., 1964). Liver function parameters that have been altered as a result of excessive PCE exposure include sulfobromophthalein retention time, thymol turbidity, serum bilirubin, serum protein patterns, cephalin-cholesterol flocculation, serum alkaline

phosphatase, SGOT, and serum lactic acid dehydrogenase (LDH). However, these parameters may result from other causes that are completely dissociated with PCE.

Effects attributed to exposure to PCE at high or unknown levels included cirrhosis of the liver (Coler and Rossmiller, 1953), toxic hepatitis (Hughes, 1954; Meckler and Phelps, 1966), liver cell necrosis (Trense and Zimmerman, 1969; Meckler and Phelps, 1966), and enlarged liver (Meckler and Phelps, 1966). In some cases, liver dysfunction parameters returned to normal following cessation of exposure (Hughes, 1954). In one case, the liver was enlarged 6 months after cessation of exposure (Meckler and Phelps, 1966).

Chielewski et al. (1976) found that SGOT and SGPT levels were significantly elevated in a group of 16 of 25 workers compared to non-exposed controls. The group of 16 had been exposed to PCE vapors in the range of 59 to 442 ppm (400 to 3000 mg/m³) for periods ranging from 2 months to 27 years. Serum enzyme levels in the remaining group of 9 who were exposed to levels of PCE at or below 29 ppm (200 mg/m³) were normal. Low urinary excretion of 17-ketosteroids and abnormal EEG tracings was reported for members of both exposed groups, but no cause-effect relationship to PCE exposure can be inferred.

6.1.2 Effects on Kidneys

Associations between high air levels of PCE and symptoms of renal dysfunction have been reported by Larsen et al. (1977) and Hake and Stewart (1977). Symptoms included diminished excretion of urine, uremia, elevated serum creatinine (Larsen et al., 1977), and proteinuria and hematuria (Hake and Stewart, 1977). A cause-effect relationship with PCE exposure cannot be inferred from the report by Larsen et al. (1977). Similarly, other factors that may have been responsible for the observation of renal damage in the case report by Hake and Stewart (1977) cannot be precluded.

6.1.3 Effects on Other Organs/Tissues

6.1.3.1 Effects Upon the Pulmonary System and Skin--Overexposure to high but unknown concentrations of PCE have been associated with pulmonary damage (Patel et al., 1977; Levine et al., 1981).

Effects of PCE upon the skin range from a mild to moderate burning sensation upon direct contact for 5 to 10 minutes, to a marked erythema after prolonged exposure, and finally, blistering if PCE is trapped under clothing

or in shoes (Hake and Stewart, 1977). Observations of this nature have been reported also by Stewart and Dodd (1964), Morgan (1969), and Ling and Lindsay (1971).

6.1.3.2 Effects Upon the Heart--In the case report of Abedin et al. (1980), work-related exposure of an individual to PCE was apparently the principal factor leading to dizziness, headaches, and premature ventricular beats. The individual's prior history showed no other possible contributing factors. The individual's symptoms were more pronounced when the PCE plasma level was high (3.8 ppm). Removal from exposure to PCE was reported to relieve the symptoms as well as the premature ventricular beats.

6.1.4 Effects on CNS and Behavior

6.1.4.1 Effects of Short-Term Exposure--Reports of accidental short-term exposure to PCE have implied that such exposure produces temporary central nervous system (CNS) effects (Coler and Rossmiller, 1953; Lob, 1957; Eberhardt and Freundt, 1966; Gold, 1969; Stewart, 1969; Bagnell and Ellenberger, 1977). Effects most prominently mentioned were dizziness, confusion, headache, nausea, and irritation of eyes and mucous tissue. Higher-level exposures intensified the above symptoms and, at sufficiently high levels, produced unconsciousness and eventually death.

An early report of short-term experimental exposures to PCE of four subjects (Carpenter, 1937) described subjective effects similar to those listed above. He also observed signs of increased autonomic activity such as palmar sweating, salivation, and nasal secretion. Such symptoms were observed at 500 ppm (3390 mg/m³) PCE exposure for about 2 hours. Concentrations of 1000 to 1500 ppm (6780 to 10,170 mg/m³) PCE for up to 2 hours produced faintness. Higher concentrations produced faintness more quickly.

Rowe et al. (1952) collected subjective reports from six subjects exposed to PCE concentrations ranging from about 100 to 1000 ppm (678 to 6780 mg/m³). No effects were observed at 100 ppm (678 mg/m³), but the odor was noticeable. The threshold for eye irritation, dizziness, and sleepiness was less than 200 ppm (1356 mg/m³) for 20 to 30 minutes. Exposure to 280 ppm (1898 mg/m³) intensified the symptoms and also produced "lightheadedness" and numbness about the mouth. Subjects reported feelings of motor incoordination at 600 ppm (4068 mg/m³) exposure levels after 10 minutes. One minute of exposure at 1000 ppm (6780 mg/m³) produced all of the above symptoms and was considered intolerable by the subjects.

Stewart et al. (1970) collected subjective reports and a few objective measures of CNS function in 11 subjects exposed to 100 ppm (678 mg/m^3) for 7 hours. This single-day study was part of a long-term exposure experiment. Symptoms reported were headache, mucous tissue and eye irritation, autonomic symptoms such as flushed skin, dizziness, sleepiness, and speech difficulty. Objective data collected were performance of the Romberg test of balance, the Flanagan test of coordination, an arithmetic test, a visual inspection task, a visual acuity test, and a test of depth perception. Of the objective tests, none showed any abnormality except for the Romberg test, which was abnormal in three of the 11 subjects. No control group was used and data were compared to known norms. Stewart et al. (1977) executed another long-term repeated exposure study of PCE using 12 subjects exposed to 0, 25, or 100 ppm (0, 169, or 678 mg/m^3) PCE. Data collected concerned the Romberg test of balance, the Michigan eye-hand coordination test, rotary pursuit performance, Flanagan coordination test, eye saccade velocity measurement, the Lorr-McNair mood scale, and a divided attention task involving the simultaneous detection of one of an array of peripheral red lights while simultaneously counting the occurrence of a white central light. EEG spectra were also collected. No effect was noted on any of these tests on the first day of exposure to 100 ppm (678 mg/m^3) PCE. In this study, data were compared to control (0 or 25 ppm PCE) days. This should have made the tests more sensitive. It is difficult to explain why this experiment using 100 ppm (678 mg/m^3) PCE did not show effects on the Romberg test while in Stewart et al. (1970) effects were reported. The fact that only three of the 11 subjects showed an abnormal Romberg test in Stewart et al. (1970) might indicate abnormally sensitive subjects or the occurrence of chance results.

Only three of the above publications (Rowe et al., 1952; Stewart et al., 1970; Stewart et al., 1977) reported sufficiently quantitative data at lower exposure limits so that CNS threshold effects can be estimated. It appears that such effects as dizziness, eye and mucous tissue irritation, headache, and sleepiness all appear in mild form when humans are exposed to between 100 and 200 ppm (678 and 1356 mg/m^3) PCE. Higher exposure concentrations produce intensified symptoms leading to confusion and nausea. Feelings of motor incoordination and faintness occur between 600 and 1000 ppm (4068 and 6780 mg/m^3). The data are too scant to hazard a generalization about the durations required to produce symptoms. The above symptoms were reported at times ranging from 10 minutes to 2 hours. All symptoms due to experimental exposures

were reversed in a matter of hours after cessation of exposure. These conclusions should be treated with extreme skepticism, however, due to the extremely small data base, the few subjects studied, and the largely qualitative methods employed.

6.1.4.2 Effects of Long-Term Exposures--Data about long-term (greater than a few weeks) exposure to PCE in humans originate entirely from case studies (Grossdorfer, 1952; Coler and Rossmiller, 1953; Lob, 1957; Gold, 1969; Weichardt et al., 1975). Extended exposure to PCE appears to make many of the temporary symptoms produced by short-term exposure more continuous or of a longer duration. Reports of frequent dizziness, headache, nausea, fatigue, and disorientation are common even for extended periods of time after cessation of exposure. New symptoms which have no clear analogs in the short-term case appear, however, during long-term exposure. Long-term exposed subjects are reported to have short-term memory deficits, ataxia, irritability, disorientation, sleep disturbances and decreased alcohol tolerance. Such symptoms are sometimes reported to be irreversible.

Stewart et al. (1970) and Stewart et al. (1977) performed repeated experimental exposures to 100 ppm (678 mg/m^3) PCE. While these were not long-term studies with respect to frequent industrial exposures, these studies could provide data about cumulative effects and adaptation over a moderate period of time. Subjective reports indicated that perceived odor intensity decreased over repeated exposures as well as within days. Subjective complaints also decreased over the course of repeated exposures. No systematic trend was found in objective test results (see Section 6.1.4.1 for details), and indeed it may be argued that no effects on objective test results could be demonstrated due to PCE exposure at all.

Stewart et al. (1977) conjectured that repeated PCE exposure effects might be exacerbated by simultaneous administration of either alcohol or Diazepam. While both alcohol and Diazepam produced decrements in the various objective tests (see Section 6.1.4.1 for details), the effects were no worse when these substances were combined with PCE exposure. It would appear that repeated 100-ppm (678 mg/m^3) PCE exposure is not close to the threshold for objective test effects.

There is only limited useful information concerning the effects of long-term exposure to PCE. Case studies provide poor exposure data and are potentially confounded with exposure to other similar substances which would be expected to produce similar results, e.g., Tuttle et al. (1977). Control

groups are frequently absent in such studies as well. From repeated exposures in a laboratory setting (Stewart et al., 1970; Stewart et al., 1977) it is apparent that some sensory and symptom adaptation to low-level PCE exposure occurs, but the extent to which such adaptation occurs at higher levels and the sequelae of such adaptation are not known.

6.2 LABORATORY ANIMAL STUDIES

Reported effects associated with PCE exposure of laboratory animals include effects on the CNS, cardiovascular system, liver, kidney, skin, eyes, and the immune system. A summary of effects and dose data are provided in Tables 6-1 and 6-2.

6.2.1 Lethality and Anesthesia

The lethality and anesthesia levels for single exposures to PCE in rats were studied by Carpenter (1937) and Rowe et al. (1951). Data reported by the above articles were used to construct the curves of Figure 6-1. Linear regression analyses were used to predict the time required to produce an effect for various concentrations of PCE in a log-log plot. The line labeled "LC100" represents the time required to just produce death at various concentrations of PCE. The line labeled "ANESTHESIA" is the time required to just produce anesthesia at various concentrations of PCE. The "LC0" line is the prediction line for the time required to produce effects just short of death at various concentrations of PCE. In order to depict the time by concentration curve for anesthesia in linear space, Figure 6-2 was plotted. It appears that anesthesia may be produced in rats by sufficiently long single exposures even by concentrations of PCE as low as 2500 ppm (16,950 mg/m³), even though nearly 16 hours of exposure is required. While the curves in Figures 6-1 and 6-2 are probably of the correct form, the exact values are likely to be in error. Data were fitted to reported means from groups of unequal size. There is likely to be variation due to strain of rats. Log transformed data were antilog transformed to produce Figure 6-2, a process which does not necessarily produce a least squares fit in linear space.

Repeated exposure to higher concentrations of PCE appear to produce considerable tolerance in rats. Carpenter (1937) reported that 2,750 ppm (18,645 mg/m³) PCE produced anesthesia in rats on the first exposure day. After six such exposures, however, rats did not become anesthetized even though 10,000 ppm (67,800 mg/m³) exposures were tried. This finding corresponds to reports

TABLE 6-1. SUMMARY OF THE EFFECTS OF PCE ON ANIMALS

Animal species	Dose concentration	Route of administration	Exposure variables	Effects	Reference
Rabbit (female)	N/A	skin	single application single instillation (eye)	primary eye and skin irritant	Duprat et al., 1976
Rabbit	13 mmol/kg	oral	single dose	marked increase in serum enzymes, i.e., alkaline phosphatase, SGOT, and SGPT within 24 hours	Fujii et al., 1975
Mouse	200 ppm	inhalation	4 hours <u>single exposure</u>	moderate fatty infiltration of the liver 1 day after exposure but not 3 days after	Kylin et al., 1963
Mouse	200 ppm	inhalation	4 hours/day 6 days/week 1-8 weeks	fatty degeneration of the liver	Kylin et al., 1965
Guinea pig	100 ppm	inhalation	7 hours/day 5 days/week 132 exposures	increased liver weights in females	Rowe et al., 1952
Guinea pig	200 ppm	inhalation	7 hours/day 5 days/week	increased liver weights with some fatty degeneration in both males and females - slight increase in lipid content and several small fat vacuoles in liver	Rowe et al., 1952
Guinea pig	400 ppm	inhalation	7 hours/day 5 days/week 169 exposures	more pronounced liver changes than at 200 ppm, slight cirrhosis was observed - increased liver weight, increase in neutral fat and esterified cholesterol in the liver, moderate central fatty degeneration, cirrhosis	Rowe et al., 1952

TABLE 6-1. (continued)

Animal species	Dose concentration	Route of administration	Exposure variables	Effects	Reference
Guinea pig	2500 ppm	Inhalation	18 7-hour exposures	loss of equilibrium, coordination, and strength, increase in weights of liver and kidney, fatty degeneration of the liver, cloudy swelling of tubular epithelium of the kidney	Rowe et al., 1952
Rabbit Rat Monkey	100-400 ppm	Inhalation	7 hours/day 5 days/week 6 months	no abnormal growth, organ function, or histopathologic findings	Rowe et al., 1952
Rabbit	2500 ppm	Inhalation	28 7-hour exposures	central nervous system depression without unconsciousness	Rowe et al., 1952
♂ Rat	2500 ppm	Inhalation	1-13 7-hour exposures	loss of consciousness and death	Rowe et al., 1952
Rat	1600 ppm	Inhalation	18 7-hour exposures	drowsiness, stupor, increased salivation, extreme restlessness, disturbance of equilibrium and coordination, biting and scratching reflex	Rowe et al., 1952
Rat	3000-6000	Inhalation	single exposure up to 8 hours	increase in liver weight, increase in total lipid content of liver accompanied by a few diffusely distributed fat globules	Rowe et al., 1952
Rabbit	15 ppm	Inhalation	3-4 hours/day 7-11 months	depressed agglutinin formation	Mazza, 1972
Rabbit	2212 ppm (15 mg/L)	Inhalation	45 days 4 hrs/day 5 days/week	liver damage indicated by elevated SGPT, SGOT, SGLDH; marked reduction of Schmidt index	Mazza, 1972

TABLE 6-1. (continued)

Animal species	Dose concentration	Route of administration	Exposure variables	Effects	Reference
Rat	70 ppm	inhalation	8 hours/day 5 days/week 150 exposures (7 months)	no pathological findings	Carpenter, 1937
Rat	230 ppm	inhalation	8 hours/day 5 days/week 150 exposures (7 months)	similar, but less severe, pathological findings as with 470 ppm - congestion and light granular swelling of kidneys	Carpenter, 1937
Rat	470 ppm	inhalation	8 hours/day 5 days/week 150 exposures (7 months)	congested livers with cloudy swelling, no evidence of fatty degeneration or necrosis: evidence of kidney injury - increased secretion, cloudy swelling, and desquamation of kidneys: congestion of spleen	Carpenter, 1937
Rat	2750-9000 ppm	inhalation	single exposure	no deaths	Carpenter, 1937
Rat	19000 ppm	inhalation	30-60 minutes	congested livers with granular swelling, some deaths	Carpenter, 1937
Rabbit	15 ppm	inhalation	3-4 hours/day 7-11 months	moderately increased urinary urobilinogen, pathomorphological changes in the parenchyma of liver and kidneys	Navrotskii et al., 1971
Rabbit	2211 ppm (15 mg/L)	inhalation	45 days	significant reduction of glomerular filtration rate and the renal plasma flow; decrease of highest excretory tubular capacity (kidney damage)	Brancaccio et al., 1971

TABLE 6-1. (continued)

Animal species	Dose concentration	Route of administration	Exposure variables	Effects	Reference
Mouse (Swiss male, 10 animals)	2.5 mL/kg	intraperitoneal		100 mg percent or more protein found in one of six mice - proximal convoluted tubules were swollen in all animals and necrotic in one	Plaa and Larson, 1965
Mouse (10 Animals)	5.0 mL/kg	intraperitoneal		two of four mice had 100 mg percent or more protein in urine	
Rabbit	2211 ppm (15 mg/L)	Inhalation	(urine samples were collected 24 hours post-injection) 45 days	increased plasma and urine levels of adrenal cortical and adrenal medullar hormones; increased excretion of principal catecholamine metabolite (not statistically significant)	Mazza and Brancaccio, 1971
Mouse	N/A	intraperitoneal		liver dysfunction	Klaassen and Plaa, 1966
Mouse	N/A	intraperitoneal		LD ₅₀	Klaassen and Plaa, 1966
Dog		intraperitoneal		elevated SGPT	Klaassen and Plaa, 1967
Dog		intraperitoneal		caused phenolsulfonaphthalein retention indicating kidney dysfunction	Klaassen and Plaa, 1967
Dog		intraperitoneal		LD ₅₀	Klaassen and Plaa, 1967
Rat	300 ppm	Inhalation	7 hours/day days 6-15 of gestation	decreased maternal weight gains, increased fetal reabsorptions	Schwartz et al., 1975

TABLE 6-1. (continued).

Animal species	Dose concentration	Route of administration	Exposure variables	Effects	Reference
Mouse	300 ppm	inhalation	7 hours/day days 6-15 of gestation	maternal liver weights increased relative to body weight; increased incidences of fetal subcutaneous edema, delayed ossification of skull bones, and split sternbrae	Schwartz et al., 1975
Rat	44.2 ppm	inhalation	entire gestation period	decreased levels of DNA and total nucleic acids in the liver, brain, ovaries, and placenta	Aninina, 1972
Mouse	15-74 ppm	inhalation	5 hours/day 3 months	decreased electroconductance of muscle and "amplitude" of muscular contraction	Dmitrieva, 1968
Rat	15 ppm	inhalation	4 hours/day 5 months	EEG changes and protoplasmic swelling of cerebral cortical cells, some vacuolated cells and signs of karyolysis	Dmitrieva, 1966
Rat	73 and 147 ppm	inhalation	4 hours/day 4 weeks	EEG and electromyogram changes; decreased acetylcholinesterase activity	Dmitrieva, 1966
Dog (male beagles)	0.5-1.0% v/v 5000 & 10000 ppm	inhalation	7 minutes house air followed by 10 minutes of tetrachlorethylene 8 µg/kg epinephrine given I.V. (1) a control dose after 2 minutes of breathing air (2) challenge dose after 5 minutes of breathing test compound	cardiac sensitization (development of serious arrhythmia or cardiac arrest) was not induced at the concentrations tested (other similar compounds gave positive results at same concentration,	Reinhardt et al., 1973

TABLE 6-1. (continued)

Animal species	Dose concentration	Route of administration	Exposure variables	Effects	Reference
Cat	3000 ppm	inhalation	4 hours	no anesthesia	Lehmann, 1911
Cat	14,600 ppm	inhalation	1-2 hours	anesthesia	Lehmann and Schmidt-Kehl, 1936
Mouse	40 mg/L 5,900 ppm	inhalation		minimal fatal concentration	Lamson et al., 1929
Mouse	4-5 mL/kg	oral		death in 2-9 hours from central nervous system depression	Lamson et al., 1929
Rabbit	5 mL/kg	oral (in oil)		death in 17-24 hours	Lamson et al., 1929
Cat	4 mg/kg	oral (in oil)		death within hours	Lamson et al., 1929
Dog	9000 ppm	inhalation		narcosis, marked salivation, "narrow margin of safety"	Lamson et al., 1929
Dog	4-25 mL/kg	oral (in oil)		death in 5-48 hours	Lamson et al., 1929

TABLE 6-2. TOXIC DOSE DATA

Description of exposure ^a	Species	Route of administration	Dose concentration	Toxic effect endpoint	Time	Reference
LD ₅₀	mouse (male)	oral	8100 mg/kg	death	36 hr	Wenzel and Gibson, 1951
LD ₅₀	mouse	interperitoneal	2.9 mL/kg 28 mM/kg 4700 mg/kg	death	24 hr	Klaassen and Plaa, 1966
ED ₅₀	mouse	interperitoneal	2.9 mL/kg 28-32 mM/kg	liver dysfunction		
LD ₅₀	mouse	interperitoneal	34 mM/kg	death	24 hr	Gehring, 1968
ED ₅₀	mouse	interperitoneal	24 mM/kg	liver toxicity		
LD ₅₀	dog	interperitoneal	2.1 mL/kg 21 mM/kg 3400 mg/kg	death	24 hr	Klaassen and Plaa, 1967
ED ₅₀	dog	interperitoneal	0.74 mL/kg 7.2 mM/kg	liver damage	24 hr	Klaassen and Plaa, 1967
ED ₅₀	dog	interperitoneal	1.4 mL/kg	kidney dysfunction	24 hr	Klaassen and Plaa, 1967
LD ₅₀	mouse	subcutaneous	390 mM/kg	death	10 days	Plaa et al., 1958
ED ₅₀	mouse	subcutaneous	27 mM/kg	liver toxicity		Plaa et al., 1958
LD ₅₀	mouse	oral (undiluted)	0.109 mL	death	unknown	Dybing and Dybing, 1946
LD ₅₀	mouse	oral (in oil)	0.134 mL	death	unknown	Dybing and Dybing, 1946
LD ₅₀	mouse	oral	8850 mg/kg	death	unknown	Handbook of Toxicology, 1959
LCL ₀	mouse	inhalation	23000 mg/m ³	death	2 hr	
LD ₅₀	rat			death		Withey and Hall, 1975

TABLE 6-2. (continued)

Description ^a of exposure	Species	Route of administration	Dose concentration	Toxic effect endpoint	Time	Reference
LCL ₀	rat	inhalation	4000 ppm	death	4 hr	Handbook of Toxicology, 1959
LCL ₀	rat	inhalation	4000 ppm	death	4 hr	
LDL ₀	dog	oral	4000 mg/kg	death	unknown	Arch. Hyg. Bakteri- ol. 116:131, 1936
LDL ₀	dog	intravenous	85 mg/kg	death	unknown	Carpenter et al., 1949
LDL ₀	cat	oral	4000 mg/kg	death	unknown	Clayton, 1962
LDL ₀	rabbit	oral	5000 mg/kg	death	unknown	Lamson et al., 1929

^aLCL₀ - the lowest concentration of a substance, other than an LC₅₀, in air which has been reported to have caused death in humans or animals.

LDL₀ - the lowest dose of a substance, other than an LD₅₀, that is introduced by any route other than inhalation over any given period of time and reported to have caused death in humans or animals introduced in one or more divided portions.

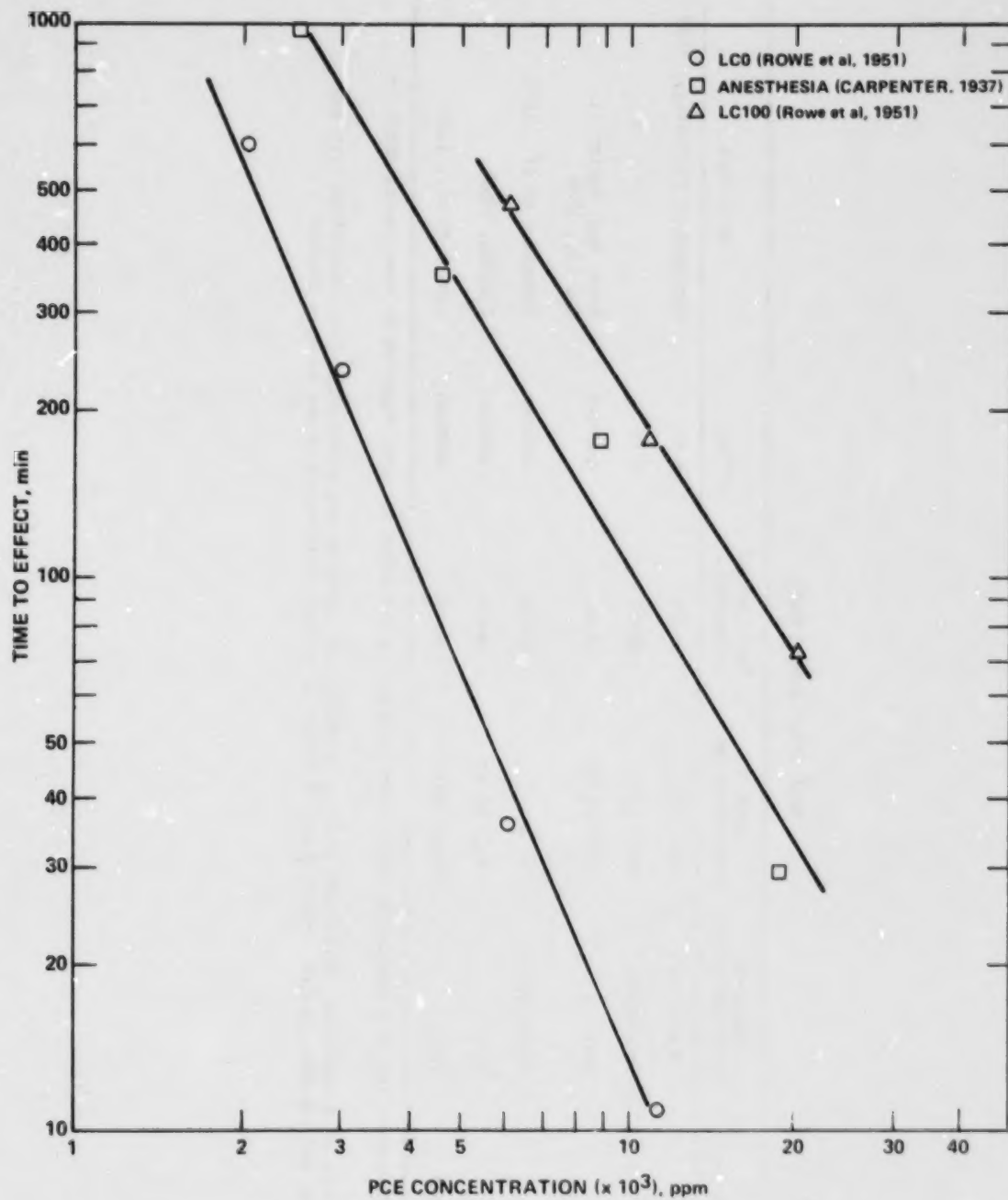


Figure 6-1. Concentration-time curves for various effects of PCE

Source: Carpenter (1937) and Rowe et al. (1951).

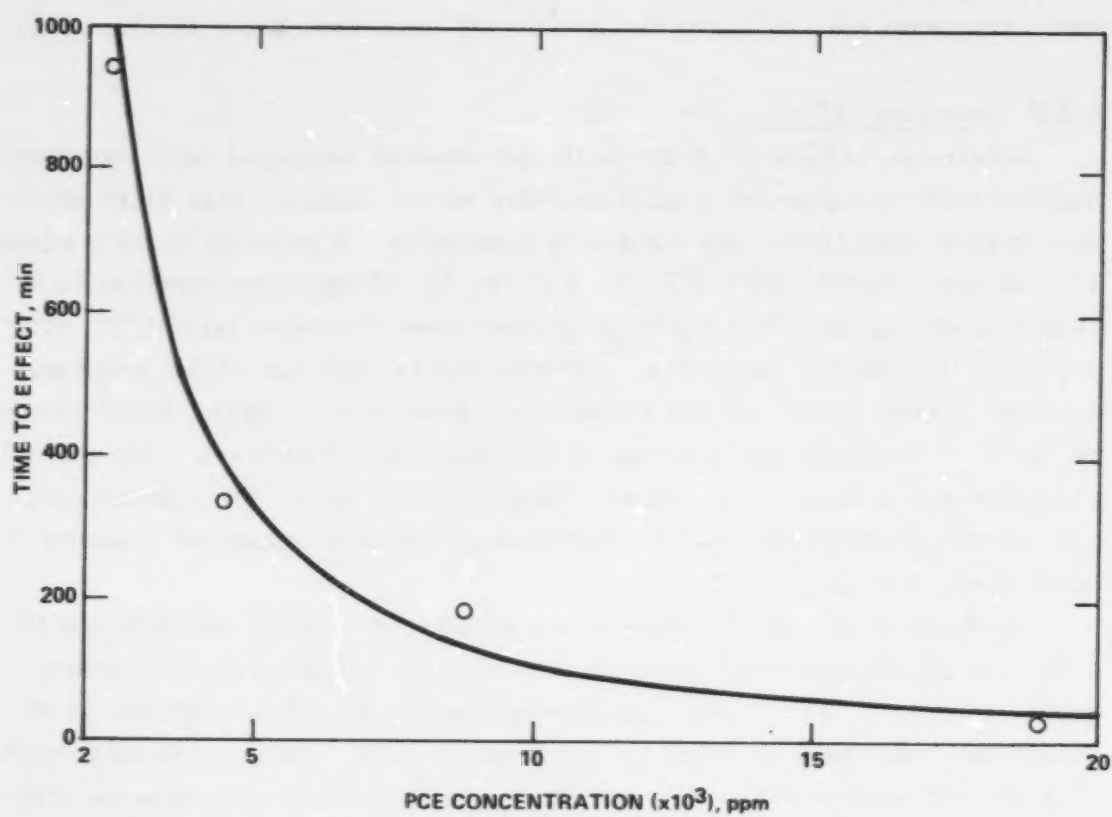


Figure 6-2. Concentration-time curve for PCE-induced anesthesia.

of tolerance in humans (Stewart et al., 1970; Stewart et al., 1977). Behavioral adaptation, although in a less obviously systematic way, has also been reported in rats by Rowe et al. (1951) and Goldberg et al. (1964) (see Section 2.2.2).

Effects of high-level PCE exposure in rats appeared to be similar to those of other anesthetics. When death occurred due to a single PCE exposure, the process was highly similar to narcotic overdose (Carpenter, 1937). Although rats dying from repeated exposures to high-level PCE showed some liver involvement, the death was still attributable to CNS impairment (Rowe et al., 1951).

6.2.2 Behavioral Effects

Behavioral effects of high-level and repeated exposures were reported qualitatively by Carpenter (1937) and Rowe et al. (1952). Most often noted were ataxia, somnolence, and eventually anesthesia. A group of 18 rats exposed to 1600 ppm (10,848 mg/m³) PCE for 7 hr/day for 18 days were reported to be stuporous during the first week and to have shown increased salivation, motor activity, "reflexive" aggression and some ataxia, but the latter symptoms subsided toward the end of the second week (Rowe et al., 1951). Brief recurrence of the symptoms was reported at the beginning of each week, but overall reduction was a function of repeated exposures. Some of the symptoms are reminiscent of those reported in high-level, long-term industrial exposure of humans (see Section 2.1.4.2).

Goldberg et al. (1964) studied the effects of 0, 1150, and 2300 ppm (0, 7797, and 15,594 mg/m³) PCE exposure for 4 hr/day, 5 days/week for 2 weeks, in groups of 8 to 10 rats. Rats were tested before and after exposure on an escape/avoidance task in which the response was pole climbing. An 80 percent loss of both escape and avoidance responses due to ataxia was reported after the first 2300 ppm (15,594 mg/m³) exposure period. Upon repeated exposures, however, the effect was diminished. No data were presented on the adaptation effect other than that it occurred.

Low-level repeated PCE exposure (200 ppm, 6 hr/day, for 4 days) was studied in groups of 10 rats by Savolainen et al. (1977). Many dependent measures were collected, including brain protein, RNA, glutathione, acid proteinase, and nonspecific cholinesterase and six aspects of open field behavior. The behavioral variables were collected and a separate significance test was done on each measure after the last exposure day and 17 hours later. Of the 12 t-tests performed, only the preening rate and preening time were significant and only immediately after the last exposure. The authors also

discussed reduced RNA and increased nonspecific cholinesterase content in brain due to PCE exposure. If these results are replicable, they represent a near threshold effect of PCE in rats.

Ataxia and somnolence due to PCE ingestion have been reported in dogs given 0.22 mg/kg for worm treatment (Snow, 1973; Myer and Jones, 1954). However, such side effects are not universally found at this dosage (Miller, 1966; Lamson et al., 1929; Schlingman, 1925). Occurrence of neurotoxic effects probably depends upon the extent of absorption of PCE via ingestions which is in turn likely to be affected by diet (Snow, 1973) as well as by the strain of dog. In the absence of tissue levels, ingestion data are difficult to interpret.

Extremely low levels of PCE exposure were reported to have affected electrophysiological responses in rats (Dmitrieva, 1966, 1968, 1973; Dmitrieva et al., 1968). Documentation is sparse, and the effective dose levels are so low compared to the rest of the literature that the credibility of the reports must be questioned until independently replicated.

6.2.3 Effects on the Liver and Kidney

PCE is generally regarded as being both hepatotoxic and nephrotoxic in laboratory animals when exposure is excessive and prolonged.

Carpenter (1937) exposed three groups of 24 albino rats each to PCE vapor concentrations averaging 70, 230, or 470 ppm (475, 1560, or 3188 mg/m³) for 8 hr/day, 5 days/week, for up to 7 months. The maximum exposure for any animal during the 7-mo period was 150 days (1200 hours). A group of 18 unexposed animals served as controls.

The rats exposed to 470 ppm (3188 mg/m³) for 150 days followed by a 46-day rest period developed cloudy and congested livers with swelling; there was no evidence of fatty degeneration or necrosis. These rats also had increased renal secretion with cloudy swelling and desquamation of kidneys, as well as congested spleens with increased pigment. The pathologic changes were similar but less severe in the rats exposed to 230 ppm PCE (1560 mg/m³). In some instances, there was congestion and light granular swelling of the kidneys after 21 exposure days. After 150 days of exposure, followed by a 20-day rest, congestion was found in the kidney and spleen. The livers showed reduced glycogen storage. Microscopic evidence of damage to liver, kidney, or spleen in rats exposed at 70 ppm (475 mg/m³) for 150 days was not observed. In addition, microscopic examination of heart, brain, eye, or nerve tissue did not reveal any damaging effects in any of the chronically exposed rats. Functional

parameters, including icteric index, Van den Bergh test for bilirubin, and blood and urine analysis, were normal after the exposures. Fertility of female rats, as measured by a fertility index (actual number of litters/possible number of litters), was increased slightly after repeated exposures to 230 or 470 ppm PCE (1560 or 3188 mg/m³). No deaths or signs of anorexia or diminished activity were observed during the chronic exposures.

Carpenter also tried to determine the highest concentration of PCE vapor that would not anesthetize rats exposed for 8 hours. Exposure to 31,000 ppm (210,273 mg/m³) was lethal within a few minutes. Rats exposed to 19,000 ppm (128,871 mg/m³) died after 30 to 60 minutes. Animals that were exposed to 19,000 ppm (128,877 mg/m³) and removed from the inhalation chamber just prior to unconsciousness developed congestion and granular swelling of the liver. Similar liver effects were seen after exposure at 9000 ppm (61,047 mg/m³). There was also marked granular swelling of the kidneys. A single exposure at 9000, 4500, or 2750 ppm (61,047, 30,523, or 15,261 mg/m³) did not cause death to any of the rats in this study; however, post-mortem examinations of the rats exposed to those concentrations revealed only a slight increase in the prominence of liver and kidney markings.

Rowe et al. (1952) exposed rabbits, monkeys, rats, and guinea pigs to PCE vapor for 7 hours, 5 days/week, for up to 6 months. Exposure concentrations ranged from 100 to 2500 ppm (678 to 16,957 mg/m³). Three of the four species tested -- rabbits, monkeys, and rats -- showed no effects of repeated exposures to concentrations up to 400 ppm (2713 mg/m³). There were no adverse effects on growth, liver weight, or lipid content, or gross or microscopic anatomy observed in any animal. In contrast, guinea pigs showed marked susceptibility to PCE in this study. The liver weights of female guinea pigs increased significantly after 132 7-hr exposures at 100 ppm (678 mg/m³). At 200 ppm (1356 mg/m³), there was a slight depression of growth in female guinea pigs and increased liver weights in both males and females. Slight to moderate fatty degeneration of the liver also was observed. These effects were more pronounced in guinea pigs that received 169 7-hr exposures at 400 ppm (2713 mg/m³). At this concentration, there also were increased amounts of neutral fat and esterified cholesterol in livers. Gross and microscopic examination of the tissues revealed slight to moderate fatty degeneration in the liver with slight cirrhosis. Rowe et al. stated that at 395 ppm (2680 mg/m³), increased kidney weights also were observed in guinea pigs but not in other species. Guinea pigs have been shown to be extremely sensitive to toxicity testing.

Klaassen and Plaa (1967) showed that near-lethal i.p. doses of PCE in mongrel male and female dogs can produce damage to the kidney and liver. They estimated the ED₅₀ (effective dose in 50 percent of the animals tested) for liver and kidney damage as well as the 24-hr LD₅₀ value (lethal dose in 50 percent of the animals treated). The ED₅₀ values were measured by sulfobromophthalein, SGPT, glucose, protein, and phenolsulfonephthalein (PSP), indicators of liver or kidney dysfunction. The LD₅₀ was 2.1 mL/kg (21 mmol/kg) and the ED₅₀ for elevation of SGPT was 0.74 mL/kg (7.4 mmol/kg). The ED₅₀ for diminution of PSP excretion was 1.4 mL/kg (14 mmol/kg). After administration, effects on the liver and kidneys were determined by microscopic examination. At near-lethal doses, PCE produced moderate neutrophilic infiltrations in the sinusoids and portal areas; necrosis was not observed. Near the ED₅₀, vacuolization of centralobular hepatocytes in about half the animals was observed. After a single i.p. injection at 0.75 x LD₅₀, SGPT peaked at 2 days and rapidly declined throughout the 9-day measurement period. Kidney dysfunction was deemed significant when PSP excretion was less than 39 percent (determined 24 hr after interperitoneal injection). At near PSP excretion--ED₅₀ doses, only mild dilation of the collecting ducts was seen in some of the kidneys.

In a similar study using Swiss-Webster mice, Klaassen and Plaa (1966) determined that the 24-hour LD₅₀ was 2.9 mL/kg (28 mmol/kg). The ED₅₀ for BSP retention was 2.9 mL/kg (32 mmol/kg). The ED₅₀ for elevation of SGPT was 2.9 mL/kg (28 mmol/kg). When ethanol (5 g/kg) was administered by gavage for 3 days prior to injection of 1.0 mL/kg PCE, neither PSP excretion nor BSP retention was significantly altered. At BSP-ED₅₀, mice exhibited predominantly inflammatory changes with trace to marked quantities of lipid accumulation.

A subcutaneous injection (0.4 mL daily, for 8 weeks) of PCE to groups of rats that were fed a diet containing high protein had less of an adverse effect upon the liver than PCE alone (Dumitrache et al., 1975). PCE was observed to induce liver hypertrophy compared to the reaction of the controls ($p < 0.001$). Hypertrophy was most pronounced in the low-protein group. In treating rats fed a diet containing low protein, cholesterol ($p < 0.001$) and total liver lipids ($p < 0.001$) were elevated compared to the reaction of the controls. Twelve rats were used in each of the four groups.

Effects upon the livers of rats exposed continuously for 3 months to 0.7 and 2.7 ppm (4.5 and 19 mg/m³) were reported by Bonashevskaya (1977). No significant histomorphological changes in the liver were reported at either

exposure level. Slight alterations in succinic dehydrogenase and glucose-6-phosphate dehydrogenase were reported.

Kylin et al. (1963) noted moderate fatty degeneration of the liver with a single 4-hr exposure to 200 ppm PCE (1356 mg/m^3) in female albino mice that were sacrificed 1 day after exposure. Degeneration was not observed in mice sacrificed 3 days after exposure. The mice were exposed to PCE concentrations of 200, 400, 800, or 1600 ppm (1356 , 2713 , 5426 , or $10,852 \text{ mg/m}^3$) for 4 hr. Tissues were studied microscopically to assess the extent of necrosis and fat infiltration of the liver. Moderate to massive infiltration was observed in mice killed 1 or 3 days after exposure at 400 ppm (2713 mg/m^3) or more, but no cell necrosis was observed even after 4 hours exposure up to 1600 ppm PCE ($10,852 \text{ mg/m}^3$). Kylin et al. (1965) exposed four groups of 20 albino mice to 200 ppm PCE (1356 mg/m^3). Each group of 20 mice was exposed for 4 hr/day, 6 days per week, for 1, 2, 4, or 8 weeks. Microscopic examinations were performed on livers and kidneys of the exposed mice and controls. Fatty degeneration was particularly marked and tended to be more severe with longer exposure to PCE. Chemical determination of the liver fat content was performed in addition to the histologic examination. Correlation between the histologically evaluated degree of fatty degeneration and the concentration of extracted fat was +0.74. Liver fat content of the exposed animals was between 4 and 5 mg/g of body weight, as compared to 2 to 2.5 mg/g for the control animals. The actual fat content of the livers did not increase with duration of exposure as did the extent of the fatty infiltration. No liver cell necrosis was observed. No effect on the kidneys was reported upon histologic examination.

Mazza (1972) exposed 15 male rabbits for 4 hr/day, 5 days a week, for 45 days, to 2790 ppm PCE ($18,924 \text{ mg/m}^3$). Mazza looked at the effect of PCE on serum enzyme levels in an attempt to determine the specific location of initial liver injury as well as the severity of the damage to the liver. The Schmidt Index, which is the sum of SGOT and the SGPT divided by the serum glutamate dehydrogenase (GDH), was used as an indication of hepatic disorders. Enzymatic determinations were made before exposure and 15, 30, and 45 days after exposure to PCE. All three of the enzymes showed an increase in activity, but the GDH increased the most; GDH reduced the Schmidt Index from 6.70 to 1.79. Mazza concluded that this reduction indicates the prevalence of mitochondrial injury over cytoplasmic injury in the liver.

Mazza and Brancaccio (1971) exposed 10 rabbits for 4 hr/day, 5 days per week, for 45 days, to 2790 ppm PCE ($18,924 \text{ mg/m}^3$). These investigators found a moderate, but not statistically significant, increase in levels of adrenal cortical and medullar hormones--plasma and urinary corticosteroids and catecholamines--including increased excretion of 3-methoxy-1-hydroxymandelic acid, the principal catecholamine metabolite.

In another study, Brancaccio et al. (1971) exposed 12 male rabbits for 4 hr/day, 6 days/week, for 45 days, to 2280 ppm PCE ($15,465 \text{ mg/m}^3$) to look at effects on kidney function. They noted a reduction in glomerular filtration and renal plasma flow and a decrease in the maximum tubular excretion when measured upon cessation of the exposure regimen. Brancaccio et al. concluded that PCE causes kidney damage, primarily in the renal tubule. These findings agreed with earlier histological findings of Pennarola and Brancaccio (1968) in which kidney injury, following exposure to PCE, appeared to be primarily in the renal tubule.

Plaa and Larson (1965) dosed mice with PCE by i.p. injection. Ten mice received 2.5 mg/kg and 10 others received 5.0 mg/kg. Urine samples were collected from surviving mice 24 hours after the injection of PCE. Protein was found in the urine of one of the six surviving mice injected with the lower dose and in two of four survivors of the higher dose at levels of 100 or more mg percent. None of the survivors had greater than 150 mg percent glucose in the urine. The kidneys of the mice given the lower dose were examined microscopically. The proximal convoluted tubules were swollen in all animals and necrotic in one.

Cornish et al. (1973) administered from 0.3 to 2.0 ml PCE/kg i.p. to rats. SGOT levels were elevated at all dose levels; phenobarbital pretreatment did not alter the response.

Fujii (1975) observed an increase in serum enzyme activities (i.e., alkaline phosphatase, SGOT, and SGPT) within 24 hours after a single dose of 13 mmol PCE/kg given orally to rabbits. These changes in serum enzyme activities, indicative of liver damage, were mild and transient.

6.2.4 Effects on the Heart

The effects of PCE upon the heart have not been systematically investigated. Kobayashi et al. (1982) demonstrated in rabbits under urethane anesthesia and in cats and dogs under pentobarbital anesthesia that PCE can sensitize the myocardium to epinephrine, leading to ventricular arrhythmias and

premature contractions, bigeminal rhythm, and tachycardia. Rabbits were the most sensitive; doses of 0.6 µg epinephrine per kg and 5 mg PCE per kg led to tachycardia. In dogs, 20 to 40 mg PCE per kg, i.v., produced significant depressions in the rate of rise of left intraventricular pressure with the absence of significant effect upon arterial pressure. The mean lethal dose in cats was 81.4 ± 14.4 mg PCE per kg, i.v. As the authors noted, and as described in the preceding chapter, the absence of cardiac effects in humans in the literature may be largely due to the limitations on absorption of PCE by the lungs. Additionally, the levels to which individuals are ordinarily exposed both environmentally and occupationally are obviously below the threshold for arrhythmia production via endogenously produced epinephrine. Experiments on unanesthetized laboratory animals involving tasks designed to elicit endogenous epinephrine in combination with vapor levels of PCE should produce more useful information on the cardiotoxicity of PCE.

A lack of cardiotoxicity of PCE was reported by Reinhardt et al. (1973). Unanesthetized dogs (17) were exposed to PCE in air at levels of 5,000 or 10,000 ppm (33,915 or 67,830 mg/m³). No positive responses were observed. In this study, a response considered indicative of cardiac sensitization was the development of a life-threatening arrhythmia or cardiac arrest following a challenge of epinephrine.

Christensen and Lynch (1933) observed depression of the heart in 5 dogs at near-LD₅₀ oral doses.

6.2.5 Effects on the Skin and Eye

Duprat et al. (1976) have shown PCE to be a primary eye and skin irritant in rabbits. Instillation of the chemical into the eye produced conjunctivitis with epithelial abrasion. However, healing of the ocular mucosa was complete within 2 weeks. PCE had a severe irritant effect when a single application was made to the skin of the rabbit.

6.3 ADVERSE EFFECTS OF SECONDARY POLLUTANTS

The level of phosgene derived from photodecomposition of PCE is not likely to result in acute or long-term effects. In occupational settings or under certain conditions in which phosgene is directly formed at high temperatures from halocarbons (cigarette smoke, welding), sufficient warning from the generation of extremely irritating hydrogen chloride vapors would prevent exposure to harmful concentrations of phosgene.

6.4 SUMMARY OF ADVERSE HEALTH EFFECTS AND ASSOCIATED LOWEST OBSERVABLE EFFECT CONCENTRATIONS

6.4.1 Inhalation Exposure

A number of case reports describe accidental or occupational exposure to PCE. However, the duration and extent of exposure either were unknown or involved excessively high concentrations. The few controlled human studies available generally provide subjective information on effects resulting from short-term exposures at levels near 100 ppm (678 mg/m³). The observations in these studies follow the expected pattern for a non-specific anesthetic effect. Effects associated with chronic exposures, on the other hand, are available from animal experiments.

In humans, short-term effects of PCE exposure seem to occur beginning at 100 to 200 ppm (678 to 1356 mg/m³). Such effects include dizziness, confusion, nausea, headache, and irritation of the eyes and mucuous tissue. Higher levels of exposure increase the symptoms and eventually produce faintness and unconsciousness. Long-term exposures are reported to make the above symptoms more pervasive and also to affect short-term memory and produce disorientation, irritability, ataxia, and sleep disturbance. These conclusions are based on largely anecdotal data in field studies or limited laboratory experiments. Due to the paucity of experiments, the poor quality of the extant data base and the age of the data base, these conclusions should be viewed with considerable skepticism. Newer, more sensitive methods, better experimental control, more quantitative analysis, and a wider range of dependent variables could demonstrate effects at lower levels.

While suffering from the same deficiencies as those outlined for the data on humans and PCE exposure, data from laboratory animals are remarkably consonant with human effects. Effects on open field activity have been reported at 200 ppm (1356 mg/m³) exposure levels. Effects of higher level exposure seem to relate well to the findings in humans. It appears that the rat is a good behavioral model for PCE effects in man.

Investigations in rats have likened the effects of PCE to those of narcotic drugs and anesthetics. The lowest effects levels for PCE seem to be in CNS or behavioral variables. This seems to be true for either single or repeated long-term exposures. Similar conclusions are reached in a review by Annau (1981).

Effects upon the human kidney and/or liver are not well documented and appear to be confined to those exposure scenarios in which PCE levels are

exceedingly high and exposures are short in duration. Studies of experimental animals suggest that reversible changes (congestion, fatty infiltration) occur at exposure levels of about 200 ppm (Carpenter, 1937; Rowe et al., 1952; Kylin et al., 1963). The relevance of these data to the likelihood of similar effects in humans at similar levels is unknown.

Limited data involving anesthetized laboratory animals indicate that low doses of PCE, administered i.v., can sensitize the heart to i.v. challenges of epinephrine. However, it is not considered likely that PCE will exhibit this action in humans under exposure conditions normally experienced in the environment and the workplace.

6.4.2 Oral Exposure

The acute oral toxicity of PCE has been determined in rats, mice, cats, rabbits, and dogs. There are no subchronic oral exposure studies and only one chronic study--the NCI bioassay.

6.4.3 Dermal Exposure

Although PCE can be absorbed through unbroken skin, absorption was estimated to be minor (Stewart and Dodd, 1964). Toxic quantities would probably not be absorbed through this route.

6.5 REFERENCES FOR CHAPTER 6

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7. TERATOGENICITY, EMBRYOTOXICITY, AND REPRODUCTIVE EFFECTS

Because of its widespread use, PCE has been studied for teratogenic potential. Teratology studies have been performed in rats, mice, and rabbits, using doses of PCE which, in some studies, produced slight signs of maternal toxicity. Other studies in chicken embryos (Elovaara et al., 1979) have indicated that PCE disrupts embryogenesis in a dose-related manner. However, since administration of PCE directly into the air space of chicken embryo is not comparable to administration of dose to animals with a placenta, it is not possible to interpret this result in relationship to the potential of PCE to cause adverse effects in animals or humans.

The following discussion of studies subscribes to the basic viewpoints and definitions of the terms "teratogenic" and "fetotoxic" as summarized and stated by the U.S. Environmental Protection Agency (1980):

Generally, the term "teratogenic" is defined as the tendency to produce physical and/or functional defects in offspring in utero. The term "fetotoxic" has traditionally been used to describe a wide variety of embryonic and/or fetal divergences from the normal which cannot be classified as gross terata (birth defects) -- or which are of unknown or doubtful significance. Types of effects which fall under the very broad category of fetotoxic effects are death, reductions in fetal weight, enlarged renal pelvis, edema, and increased incidence of supernumerary ribs. It should be emphasized, however, that the phenomena of terata and fetal toxicity as currently defined are not separable into precise categories. Rather, the spectrum of adverse embryonic/fetal effects is continuous, and all deviations from the normal must be considered as examples of developmental toxicity. Gross morphological terata represent but one aspect of this spectrum, and while the significance of such structural changes is more readily evaluated, such effects are not necessarily more serious than certain effects which are ordinarily classified as fetotoxic--fetal death being the most obvious example.

In view of the spectrum of effects at issue, the Agency suggests that it might be useful to consider developmental toxicity in terms of three basic subcategories. The first subcategory would be embryo or fetal lethality. This is, of course, an irreversible effect and may occur with or without the occurrence of gross terata. The second subcategory would be teratogenesis and would encompass those changes (structural and/or functional) which are induced prenatally, and which are irreversible. Teratogenesis includes structural defects apparent in the fetus, functional deficits which may become apparent only after birth, and any other long-term effects (such as carcinogenicity) which are attributable to in utero exposure. The third category would be embryo or fetal toxicity as comprised of those effects which are potentially reversible. This subcategory would therefore include such effects as weight reductions, reduction in the degree of skeletal ossification, and delays in organ maturation.

Two major problems with a definitional scheme of this nature must be pointed out, however. The first is that the reversibility of any phenomenon is extremely difficult to prove. An organ such as the kidney, for example, may be delayed in development and then appear to "catch up." Unless a series of specific kidney function tests are performed on the neonate, however, no conclusion may be drawn concerning permanent organ function changes. This same uncertainty as to possible long-lasting aftereffects from developmental deviations is true for all examples of fetotoxicity. The second problem is that the reversible nature of an embryonic/ fetal effect in one species might, under a given agent, react in another species in a more serious and irreversible manner.

7.1 ANIMAL STUDIES

7.1.1 Mice

Schwetz et al. (1975) reported a study of Swiss-Webster mice exposed to PCE via inhalation at concentrations of 300 ppm (2034 mg/m^3) for 7 hours daily on days 6 through 15 of presumed gestation. Day 0 of gestation was designated the day a vaginal plug was observed. This concentration was cited as twice the maximum allowable exclusion limit for human industrial exposure, with the Threshold Limit Value (TLV)[®] of 100 ppm (678 mg/m^3). Concurrent controls were exposed to filtered air.

Following maternal Caesarean-sectioning on day 18 of gestation, all fetuses were examined for external anomalies. One-half the fetuses were examined for soft tissue malformations using a free-hand sectioning technique, and the other one-half of the fetuses were cleared, stained, and examined for skeletal malformations. One fetus in each litter was processed and examined, using histopathological techniques. Seventeen litters were examined. The pups in the exposed group were significantly smaller, as measured by decreases in fetal body weight. Also, slight but not statistically significant increases in the number of runts were observed, as well as sporadic increases in the numbers of fetuses with subcutaneous edema, delayed ossification of the skull, delayed ossification of the sternebrae, and splits in sternebrae. No other remarkable malformations were reported in fetuses. Increases in the absolute and relative mean maternal liver weights were reported. No evidence of teratogenicity of PCE was found at the concentration tested.

7.1.2 Rats

Schwetz et al. (1975) also administered PCE to Sprague-Dawley rats by inhalation (300 ppm; 2034 mg/m^3) for 7 hours daily, on days 6 to 15 of gestation. Control rats were exposed to filtered air. Day 0 of gestation was

designated as the day when spermatozoa were observed in smears of vaginal contents. Rats were sacrificed on day 21 of gestation. Caesarean sections were performed, and fetuses were examined for external malformations. Half of the fetuses in each litter were examined for soft tissue malformations and the remaining half of the fetuses were examined for skeletal malformations. One fetus from each litter was randomly selected for serial sectioning and histological evaluation. Average maternal body weight gain was slightly reduced in the rats exposed to PCE. A slight but statistically significant increase in resorption was reported in 9 of 17 PCE-exposed litters evaluated. Exposure of dams to PCE produced no effect on the average number of implantations per litter, fetal sex ratios, or fetal body measurements. No soft tissue or skeletal anomalies were reported in the offspring of rats exposed to PCE. No evidence of teratogenicity of PCE was found at the concentration tested.

Beliles et al. (1980) exposed Sprague-Dawley rats to PCE at 300 ppm (2034 mg/m³) for 7 hours daily, 5 days per week. Controls were administered filtered air. Nineteen to 24 rats were examined in this study. One-half of the rats were exposed for 3 weeks prior to mating. All rats were exposed during gestation either between days 0 through 18 or between days 6 through 18, with day 0 designated as the day when spermatozoa were observed in smears of vaginal contents. Three rats (days 6-18) died on the second day of pregestational treatment. Signs of ataxia and loss of balance were observed in all of the other rats of this group on the same day. The authors thought this response was most likely due to the high levels in the inhalation chamber during the last 2 hours of the day. Measurements taken 15 minutes before the end of the exposure showed 568 ppm (4061 mg/m³) but could have been higher previously. The maternal body weight gain in the PCE-treated rats was not statistically different from that in controls during the pregestational period. Inhibition of maternal body weight gain occurred during the first week, as well as increases in mean absolute, but not relative, kidney and liver weights. No embryotoxic effects were observed which were attributable to maternal exposure to PCE, except for delays in skeletal ossification. This effect, however, is thought to be a reversible effect and is not considered a malformation as such. No teratogenic effects were observed.

Nelson et al. (1980) evaluated the ability of PCE to elicit a behavioral teratogenic response in Sprague-Dawley rats. In a pilot dose-range finding study, groups of 3 rats were exposed by inhalation to 1800 or 3600 ppm (12,204 or 24,408 mg/m³) PCE. Narcotization was observed in dams. Therefore, a concentration of 900 ppm (6102 mg/m³) PCE was used in the pilot study. Signs of maternal toxicity such as severe reductions in average food intake and decreased average body weight gain were observed. Food consumption and body weight gain were also reduced but were not statistically significant in rats exposed to 900 ppm (6102 mg/m³) PCE on days 14 to 20 of gestation. Dams and pups exposed to 100 ppm (678 mg/m³) PCE on days 14 to 20 of gestation did not show any adverse effects as compared to the controls.

In the behavioral testing study, 102 pregnant rats were exposed to PCE by inhalation as follows:

- (1) 900 ppm, days 7-13 of gestation (N=19)
- (2) 900 ppm, days 14-20 of gestation (N=21)
- (3) sham-exposed, days 7-13 of gestation (N=13)
- (4) sham-exposed, days 14-20 of gestation (N=19)
- (5) 100 ppm, days 14-20 of gestation (N=15)
- (6) sham exposed, days 14-20 of gestation (N=15)

Seven behavioral tests were performed on days 4 through 46 postparturition, using one male and one female per litter. One group of rats, consisting of a male/female pair from each litter, was tested for ascent on a wire mesh screen and rotorod balancing. One male and one female rat per litter were tested for open-field activity, activity wheel, and avoidance conditioning. A third pair was tested for operant conditioning. Catecholamines (norepinephrine and dopamine), acetylcholine, and protein, measured in the brain tissue, were evaluated in 10 pups (no more than 2 per litter per treatment group) at birth, or at 21 days postparturition. Histopathological evaluation of brains for neuropathology was performed in an unreported number of pups.

Rats from dams exposed to 900 ppm (6102 mg/m³) PCE on days 7 to 13 of gestation, performed less well on discrete testing of ascent and rotorod tests, but only on certain days of testing. Offspring exposed to 900 ppm (6102 mg/m³) on days 14 to 20 of gestation performed less well on one test day in the ascent test, but later performed better than controls in the rotorod test, and were relatively more active than controls in the open-field tests. Acetylcholine levels were reduced in 21-day-old rats of dams exposed to 900 ppm

(6102 mg/m³) PCE. Dopamine levels were reduced in rats of dams exposed on days 7 to 13 of gestation.

In the group of rats exposed to 100 ppm (678 mg/m³) PCE during days 14-20 of gestation, no significant differences were observed between the offspring of these animals and their controls on any of the behavioral tests. The authors summarized that "there were generally few behavioral or neurochemical differences observed between offspring of animals exposed to 900 ppm (6102 mg/m³) PCE during either days 7-13 or 14-20 of gestation. When significant differences did appear, they occurred more often when the group exposed during days 14-20 of gestation was compared with its control group."

It should be noted that the field of behavioral teratology is in its early stages of development (Buelke-Sam and Kimmel, 1979), and such behavioral alterations cannot at this time be interpreted in terms of human effect. It also must be noted that these observed changes may be related to maternal toxicity and thus do not represent a direct toxic effect.

Tepe et al. (unpublished, 1982) exposed Long-Evans hooded female rats to 1000 ± 125 ppm (6780 ± 847 mg/m³) PCE vapors to ascertain if exposure before mating and during pregnancy was more detrimental to the embryo than exposure during pregnancy alone. Four treatment groups (30 rats each) were utilized in a two-by-two factorial design: exposure to PCE for two weeks (6 hours daily, 5 days per week) before mating, through day 20 of pregnancy; PCE before mating and filtered air during pregnancy; filtered air before and PCE during pregnancy; and filtered air throughout. One-half the dams in each treatment group were sacrificed on day 21 by ether anesthesia. Elevations in relative maternal liver weights and reduction in fetal body weight were observed without alteration in maternal weight gains in groups exposed during pregnancy. An excess of skeletal variations was seen in the group exposed before mating and during pregnancy; and excessive soft tissue variations (e.g. kidney dysplasia) occurred in the group exposed during pregnancy alone. These effects are consistent with embryotoxicity. Elevation in ethoxycoumarin dealkylase activity (an indicator of P₄₅₀ activity) was observed in maternal livers but not fetal livers with pregnancy exposure. Ethoxyresorufin dealkylase activity (an indicator of P₄₄₈ activity) was not elevated in maternal livers with PCE exposure and was not detectable in fetal livers. No other effects were observed in pregnant rats exposed for 6 hr/day on days 0-20 of gestation.

Manson et al. (unpublished, 1982) conducted a study on postnatal evaluation of offspring of the female rats exposed to 1000 ± 125 ppm (6780 ± 847 mg/m³)

PCE before and/or during pregnancy. The four treatment groups are those described above (Tepe et al., 1982). The purpose of the postnatal evaluation was to determine if the reduction in body weight or the excess skeletal and soft tissue variants observed in term fetuses from the Tepe et al. (1982) study persisted, and if PCE possessed transplacental carcinogenic activity or neurobehavioral toxicity. Weight gain and survival of offspring up to 18 months of age, and frequency of any gross lesions observed at 6- and 18-month autopsies, were not influenced by prenatal exposure to PCE. Neurobehavioral tests of general activity in open field tests at 10 and 20 days of age, and in running wheels from 40 to 100 days of age, did not indicate treatment-related effects. Likewise, results from the visual discrimination test on offspring from 130 to 170 days of age were negative. Prenatal exposure to PCE did not exert a detrimental effect on any of the parameters of postnatal maturation examined.

7.1.3 Rabbits

Beliles et al. (1980) also investigated the teratogenic potential of PCE in rabbits. Fifteen to 22 rabbits were used in this study. The rabbits were divided into six groups as follows:

Group	Conc (ppm)	Days of Exposure	
		Pregestational	Gestational
1	0 (control)	none	0-21
2	0 (control)	5 days/week; 3 weeks	0-21
3	500	none	0-21
4	500	5 days/week; 3 weeks	0-21
5	500	none	0-21
6	500	5 days/week; 3 weeks	0-21

After the pregestational exposure period was complete, rabbits from each group were mated. A positive identification of spermatozoa in the vaginal canal was taken as evidence of mating and designated as day 0 of gestation. The two control groups (1 and 2) were exposed to filtered air and the exposure groups (3, 4, 5, and 6) to 500 ppm (3390 mg/m³) PCE, 7 hours per day.

Mean body weights of rabbits during pregestational and gestational exposure indicated no significant difference between controls and treated groups. Reduced food consumption during the approximate period of days 10 through 22 of gestation was observed in groups 3 and 5 and may have been related to PCE

TABLE 7-1. SUMMARY OF REPRODUCTIVE/TERATOGENIC EFFECTS OF PCE IN LABORATORY ANIMALS

Study	TCI Purity	Species	Experimental Conditions	Study	Results
Beliles et al., 1980	Technical grade 91.43%	CD rats, New Zealand rabbits	Inhalation, 500 ppm before, during gestation, and both before and after gestation.	Teratology study	Rats maternal toxicity; changes in liver weight, embryo toxicity, reduced ossification; rabbits: variations in size and color of rabbit placenta.
Elovaara et al., 1979	99% pure dissolved in olive oil	White Leghorn chick embryos	Injection into air space 5 to 10 umole/egg on days 2,3,6 of incubation, examined 14 days after incubation.	Chick egg teratology	Malformed embryos at highest dose, decreased embryo length.
Manson et al., 1982	Technical grade (Dow Chemical Company)	Long-Evans hooded rats	Inhalation, 1000 ppm before, during gestation, and both before and during gestation.	Postnatal function evaluations	No detrimental effects in offspring observed up until 8 months after birth.
Nelson et al., 1980	Technical grade 98.5% pure	Sprague-Dawley rats	Inhalation, 100 or 900 ppm on various days of gestation.	Postnatal behavioral testing	Maternal weight loss at highest dose, slight, subtle behavioral changes in offspring.
Schwetz et al., 1975	Dow-Per 99.99% pure	Sprague-Dawley rats, Swiss-Webster mice	Inhalation, 300 ppm on days 6-15 of gestation.	Teratology study	Maternal toxicity, embryo toxicity (lowered weight of mice, increased resorption in rats, subcutaneous edema in mice offspring).

TABLE 7-1. (Continued)

Study	TCI Purity	Species	Experimental Conditions	Study	Results
Tepe et al., 1982	Technical grade (Dow Chemical Company)	Long-Evans hooded rats	Inhalation, 1000 ppm before, during gestation, and both before and during gestation.	Teratology study	Maternal toxicity, embryo toxicity, depressed fetal weight, skeletal and soft tissue variations.

exposure. Placental abnormalities were reported at all exposure levels (variation in size and color); however, these were not statistically significant as tested by rank sum analysis and were thought to reflect changes in a few litters. In addition, histopathologic evaluation failed to reveal any significant change in the placenta.

7.2 SUMMARY

The mammalian animal tests performed to date do not indicate any significant teratogenic potential of PCE. On this basis, there is no evidence that suggest that the conceptus is uniquely susceptible to the effects of PCE. The anatomical effects observed primarily reflect delayed development and generally can be considered reversible. The minor behavioral changes observed probably reflect maternal nutritional deprivation rather than a direct effect of PCE. It is important to note, however, that the reversible nature of an embryonic/fetal effect in one species might, in another species, be manifested in a more serious and irreversible manner. PCE also has been implicated in producing adverse germ cell effects with alteration in sperm morphology (see Section 8.1). The potential of PCE to cause adverse effects on reproduction or the developing conceptus is based on a limited number of studies. The teratogenic potential of PCE for humans is unknown.

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8. MUTAGENICITY

The objective of this mutagenicity evaluation is to determine whether PCE has the potential to cause mutations in germ and somatic cells of humans. This qualitative assessment is based on data derived from several short-term tests that measure different types of genetic alterations: gene mutation, chromosomal aberrations, unscheduled DNA synthesis, and mitotic recombination (Table 8-1). These tests were conducted using bacteria, Drosophila, yeast, cultured mammalian cells, whole mammal systems, and cytogenetic analyses of exposed humans. Consideration will also be given to studies concerning the mutagenicity of known and expected metabolites.

8.1 GENE MUTATION TESTS

8.1.1 Bacteria

The ability of PCE to cause gene mutations in bacteria has been studied by several investigators. Many of these investigators used the Ames Salmonella/microsome test or modifications of that test. Different purities of PCE (stabilized* and low-stabilized materials) have been evaluated. (Bacterial studies are summarized in Table 8-2.)

Tetrachloroethylene is a volatile chemical, and thus, the standard Ames/Salmonella plate test, in which precautions are not taken to prevent escape of evaporated material, is not entirely suitable for its testing. Williams and Shimada (1983, sponsored by PPG Industries, Inc.), however, modified the standard plate procedure by exposing the bacteria to the test agent in a sealed chamber. In their procedure, a known volume of test chemical was added to a glass petri plate containing a magnetic bar to ensure continuous stirring for even dissipation of vapors. The chamber was initially incubated at room temperature for 20 minutes, and then exposure was continued at 37°C for 18 hours, after which the bacterial test plates were removed from the chamber, covered with lids, and incubated another 30-54 hours at 37°C.

*Stabilization is the intentional addition of material to increase the stability of tetrachloroethylene. Typically, the added stabilizers are acid and free radical scavengers (Dr. A. Philip Leber of PPG Industries, Inc., personal communication, September 1983).

TABLE 8-1. SUMMARY OF MUTAGENICITY TESTING OF TETRACHLOROETHYLENE

A. Gene Mutation Tests		Results*	References
Ames/Salmonella assay		***,-	Williams and Shimada 1983, Margard 1978, SRI International 1983, NTP 1983, Bartsch et al. 1979, Cerna and Kypenova 1977 (abstract)
Escherichia coli K12/343/113 () Multi-purpose test		-	Henschler 1977, Greim et al. 1975
Saccharomyces cerevisiae D7 reverse mutation test (ivl-1 locus)		-	Callen et al. 1980, Bronzetti et al. 1983
Drosophila sex-linked recessive lethal test		-	Beliles et al. 1980
Host-mediated tests in mice: bacteria	wk		Beliles et al. 1980, Cerna and Kypenova 1977 (abstract)
	yeast	-	Bronzetti et al. 1983
B. Chromosomal Aberration Tests			
Rat bone marrow assay		-†	Rampy et al. 1978, Beliles et al. 1980
Mouse bone marrow assay		-	Cerna and Kypenova 1977 (abstract)
Peripheral lymphocytes from exposed humans		-	Ikeda et al. 1980
Drosophila sex chromosome loss assay		-	Beliles et al. 1980
Rat dominant lethal assay		-	Beliles et al. 1980
C. Other Tests Indicative of DNA Damaging Activity			
Unscheduled DNA synthesis in WI-38		-†	Beliles et al. 1980
Hepatocyte primary culture/DNA repair test		+,-	Williams and Shimada 1983, Williams 1983
Mitotic recombination tests in Saccharomyces cerevisiae D7		+,-	Callen et al. 1980, Bronzetti et al. 1983
D. DNA Binding Studies			
Whole mice		-	Schumann et al. 1980
E. Germ Cell Tests			
Altered sperm morphology	mouse +	rat -	Beliles et al. 1980

* + designates positive; - negative; wk weak response. Dose-response relationships were not established for the reported + results or wk results.

**Although increases several fold over background were observed, the positive results are considered weak because large amounts of material were needed to elicit the responses. Positive results were only obtained using airtight chambers (except for the study by Cerna and Kypenova 1977).

†Questionable evidence for weak or borderline activity in specific data sets.

††Positive results were found with vapor phase exposure and negative results were obtained using conventional phase exposure.

TABLE 8-2. RESULTS OF BACTERIAL TESTS OF DIFFERENT PURITIES AND SOURCES OF TETRACHLOROETHYLENE

Test system/strain	Purity/source	Concentrations tested	Metabolic activation	Protocol	Reported result	Reference
Ames/Salmonella TA98, TA100, TA1535	Perchlor 200- low-stabilized 99.93% purity PPG Industries, Inc.	1% v/v for TA98, TA1538, and TA1537; 0.1 1.0, 2.5, 5.0, 7.5, and 10% for TA100 and TA1535	Aroclor- induced rat S9 mix	Gas-phase exposure in airtight chambers	Positive at 2.5% (>97% toxicity) in base-pair substitution- sensitive strain (two to tenfold increases +/- M.A. *) dose-response not established	Williams and Shimada 1983
Ames/Salmonella TA98, TA100, TA1535 TA1537, TA1538	Perchlor 230- stabilized 99.80% purity PPG Industries, Inc.	1% v/v for TA98, TA1538, and TA1537; 0.1 1.0, 2.5, 5.0, 7.5, and 10% for TA100 and TA1535	Aroclor- induced rat S9 mix	Gas-phase exposure in airtight chambers	Positive at 2.5% (>97% toxicity) in base pair substitution sensitive strains (three to ten-fold increase +/- M.A.) dose-response not established	Williams and Shimada 1983
Ames/Salmonella TA100, TA1535	High purity Perchlor low-stabilized, 99.98+% purity, PPG Industries, Inc.	0.1, 1.0, and 2.5% v/v	Aroclor- induced rat S9 mix	Gas-phase exposure in airtight chambers	Negative	Williams and Shimada 1983

*+/- M.A. designates response similar in the presence and absence of metabolic activation

(continued on the following page)

TABLE 8-2. (continued)

Test system/strain	Purity/source	Concentrations tested	Metabolic activation	Protocol	Reported result	Reference
Ames/Salmonella TA98, TA100, TA1535 TA1537, TA1538	Nonstabilized high purity Detrex Industries, Inc.	0.01, 0.05, and 0.1 ml/ plate	Aroclor- induced rat S9 mix	Standard plate test in airtight chambers	Negative	Margard 1978
Ames/Salmonella TA98, TA100, TA1535 TA1537, TA1538	Stabilized 99.84% purity Detrex Industries, Inc.	0.01, 0.05, and 0.1 ml/ plate	Aroclor- induced rat S9 mix	Standard plate test in airtight chambers	Positive (twofold increases in frameshift- sensitive strains and TA100) at 0.1 ml/plate (160 mg/plate). >90% toxicity. S9 mix increased response (10- to 17-fold increases)	Margard 1978
Ames/Salmonella TA98, TA100, TA1535 TA1537	99+% purity Aldrich	0.025, 0.05, 0.1, 0.5, 1.0 and 1.5 added to petri plate at bottom of desiccator	Aroclor-induced female and male rat liver S9 mix and Aroclor-in- duced female and male mouse liver S9 mix	Gas-phase exposure in airtight chambers	Negative	SRI Inter- national 1983
Ames/Salmonella TA98, TA100, TA1535 TA1537	Technical grade Fisher	3, 10, 33, 100, 333 ug/plate	Aroclor-induced rat liver S9 mix Aroclor-induced hamster liver S9 mix	Preincu- bation assay 10 min. 37°C	Negative	NTP 1983

(continued on the following page)

TABLE 8-2. (continued)

Test system/strain	Purity/source	Concentrations tested	Metabolic activation	Protocol	Reported result	Reference
<u>Ames/Salmonella</u> TA100	99.7% purity Merck-Darmstadt	0 to 663 mg/plate (4x10 ⁻⁵ M)	Phenobarbital- induced mouse liver microsomes with and without cofactors	Standard plate test	Negative	Bartsch et al. 1979
<u>Ames/Salmonella</u> tester strains not reported	Not reported	Not reported		Spot test	Positive	Cerna and Kypenova (1977, abstract)
<u>E. coli</u> K12/343/113 (>)	analytical grade Merck-Darmstadt	0.9 mM	Phenobarbital- induced mouse liver micro- somes	Liquid suspension 2 hours at 37°C	Negative	Greim et al. 1975
Host-mediated assay ICR mice/ <u>Salmonella</u> TA1950, TA1951, TA1952	Not reported	Not reported	Female mice	Not reported	Positive (mortality not reported)	Cerna and Kypenova (1977, abstract)
Host-mediated assay male and female CD mice/ <u>Salmonella</u> TA98	91.43% purity North Strong Division Chemicals	Inhalation at 100 ppm and 500 ppm for 5 consecutive days	Male and female mice	Bacteria in- jected intra- peritoneally after last exposure and removed 3 hours	Positive (two-fold increases in revertants from 100 ppm in males; and four-fold increases in revertants from 500 ppm females).	Beliles et al. 1980

In the Williams and Shimada (1983) study, three types of material (provided by PPG Industries, Inc.) were tested in the presence and absence of S9 mix (derived from livers of Aroclor-induced rats): Perchlor 200 (low-stabilized, 99.93 percent purity), high purity Perchlor (low-stabilized, 99.98+ percent purity), and Perchlor 230 (stabilized, 99.80 percent purity).^{*} Perchlor 200 and Perchlor 230 were evaluated in tester strains TA98, TA1537, and TA1538 at 1 percent (v/v) and in tester strains TA100 and TA1535 at 0.1, 1.0, 2.5, 5.0, 7.5, and 10 percent (v/v). High-purity Perchlor was evaluated in TA100 and TA1535 at 0.1, 1.0, and 2.5 percent (v/v). These concentrations represent the predicted concentration of the compound in the gas phase based upon calculations that consider the chamber volume and absolute temperature of the chamber, atmospheric pressure, and density of the test compound. (For example, 0.06, 0.62, 1.55, 3.1, 4.65, and 6.2 ml of PCE was added to the chamber for a 0.1, 1.0, 2.5, 5.0, 7.5, and 5 percent v/v vapor target concentration, respectively.) Positive responses were obtained for Perchlor 200 and 230 at 2.5 percent v/v (or 1.55 ml per desiccator) but not for high-purity Perchlor at the concentrations tested. A slightly higher response was observed with stabilized material. Positive results were repeated in a second experiment and were similar in the presence or absence of S9 mix. For example, in the absence of S9 mix, Perchlor 200 (at 2.5 percent) increased the number of revertant colonies in the base-pair substitution-sensitive strains TA1535 (six to tenfold increases) and TA100 (two to threefold increases); Perchlor 230 (at 2.5 percent v/v) also caused increases in revertant numbers in TA1535 (approximately tenfold increases) and TA100 (approximately threefold increases). (See Figure 8-1 for an illustration of these responses). Negative results were found in frameshift-sensitive strains (TA98 and TA1538) for both Perchlor 200 and 230 in the presence or absence of S9 mix. At the next highest concentration (5 percent v/v) the revertant counts decreased to zero as toxicity became an important factor in the tests. The authors also indicated the test materials were toxic (>97 percent killing) at the concentration (i.e., 2.5 percent v/v) that induced the mutagenic responses as determined by a simultaneous cytotoxicity test. Although

^{*}Perchlor 200 contained 0.012 (percent by weight) of hydroquinone monomethyl ether (HQMMME) which provides minimal stabilization; Perchlor 230 contained 0.011 percent HQMMME, 0.07 percent cyclohexene oxide, and 0.05 percent B-ethoxy propionitrile; high-purity Perchlor contained 0.01 percent HQMMME (written communication from Dr. A. Philip Leber of PPG Industries, Inc., 1983).

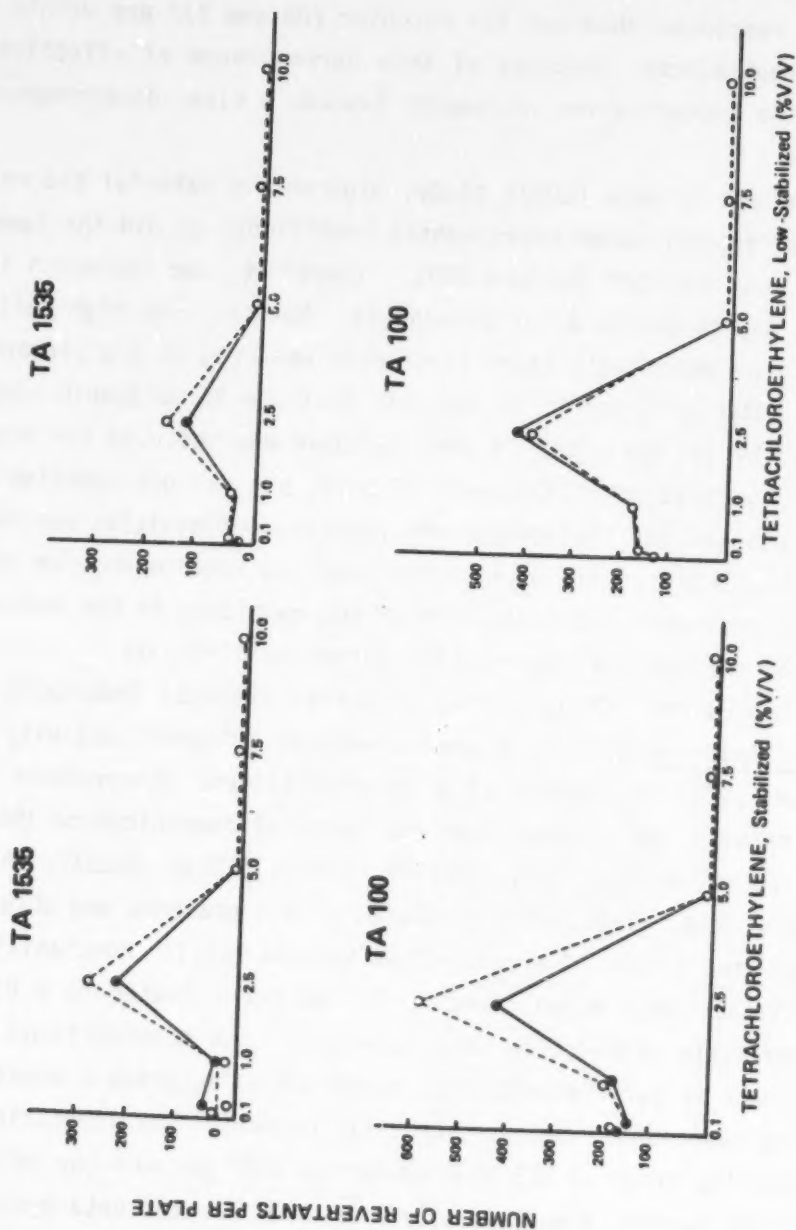


Figure 8-1. Dose-response curves for Perchlor 200 (low-stabilized tetrachloroethylene) and Perchlor 230 (stabilized tetrachloroethylene) using *Salmonella typhimurium* tester strains TA100 and TA1535 in the presence of (O---O) and in the absence of (●---●) S9 mix (150 μ l of Aroclor-induced rat liver). Each data point represents the geometric mean of triplicate plates from one experiment. (Williams and Shimada 1983)

the test materials may be quite toxic at 2.5 percent v/v, the investigator's method of quantifying cytotoxicity may not be accurate because the cell density that was used on each plate for the determination of toxicity is several orders of magnitude lower than that used for determination of mutagenicity (i.e., 10^7 - 10^9 cells). Nevertheless, it should be noted that because of toxicity, the mutagenic responses observed for Perchlor 200 and 230 are within a narrow range of concentrations. Because of this narrow range of effective concentrations and the concentration increments tested, a clear dose-response was not demonstrated.

In the Williams and Shimada (1983) study, high-purity material did not produce a detectable response under experimental conditions, as did the lower-purity materials (i.e., Perchlor 200 and 230). Therefore, the increases in revertants observed may be due to a contaminant(s). However, the high-purity Perchlor appeared to be more toxic (zero revertants observed at 2.5 percent) than the other test materials, and it is possible that a weak mutagenic response may have been masked by its toxicity. A weak response was observed for high-purity material at 1 percent in TA1535 (with S9 mix), but was not repeatable. Also, it should be pointed out that concurrent negative and positive controls were not used in this study. This weakens the negative conclusions for the high-purity material and makes interpretation of the magnitude of the responses in the presence of S9 mix for the lower-purity materials difficult.

Margard (1978) examined PCE (provided by Detrex Chemical Industries, Inc.) in the Ames/Salmonella test to determine whether mutagenic activity of technical grade samples is the result of added stabilizers. Precautions to prevent escape of material were taken (Margard, personal communication 1981), but they were not specified in writing. Tester strains TA1535, TA1537, TA1538, TA98, and TA100 were used. Tests were conducted in the presence and absence of S9 mix (prepared from livers of Aroclor-1254 induced rats). Nonstabilized and stabilized materials were added directly to the petri plates at 0.01, 0.05, and 0.1 ml per plate rather than vapor exposure. The nonstabilized test material was described as purified PCE that contained no detectable epoxides or other stabilizing components, and the stabilized material was identified as an industrial degreasing grade of PCE that contained 0.07 percent (by weight) epichlorohydrin, 0.007 percent N-methylmorpholine, 0.07 percent beta-hydroxypropionitrile, and 0.01 percent hydroquinone monoethyl ether (written communication from L. Schlossberg, Detrex Chemical Industries Inc., 1981). Nonstabilized test material was not detected as positive in the presence or absence of

S9 mix. But stabilized material at 0.1 ml per plate (equivalent to 160 mg^{*}) caused a weak response in the absence of S9 mix; twofold increases were observed for TA1538, TA98, and TA100. In the presence of S9 mix, greater increases in the number of revertants for tester strains TA1538 (17-fold increase), TA98 (10-fold increase), and TA100 (1.7-fold increase) at 0.1 ml per plate, were found. A clear dose-response was not observed for any of the tester strains. The toxicity of the test material was reported as greater than 90 percent killing at concentrations which caused increases in the number of revertants. The method used to determine toxicity may be inaccurate, as discussed previously for the Shimada and Williams study (1983). Negative results were reported for stabilized material in TA1535 and TA1537. Although the stabilized test material contained epichlorohydrin, which has been shown to be strongly mutagenic in Salmonella, it is primarily active in base-pair substitution-sensitive strains (i.e., TA1535 and TA100; McCann et al. 1975, Anderson et al. 1978), and thus it does not seem likely that this agent can solely account for the activity observed for stabilized material in the frameshift-sensitive strains TA1538 and TA98. Nevertheless, it appears that the mutagenic activity is due to the presence of a contaminant(s).

Other investigators have conducted studies in which precautions were taken to prevent escape of test material during testing. SRI International (1983 EPA-sponsored test) reported PCE (99+ percent purity, Aldrich) to be negative when tested as a vapor in sealed desiccators using TA1535, TA1537, TA98, and TA100 with or without S9 mix prepared from livers of female and male Aroclor-1254 induced rats and mice. Cells were exposed at 37°C for 8 hours in desiccators, and then incubated at 37°C for an additional 42 hours. The concentrations tested were 0.05, 0.1, 0.5, 1.0, 1.5 ml added to a petri plate at the bottom of the desiccator chamber in one experiment and 0.025, 0.05, 0.1, 0.5, and 1.0 ml added to a plate at the bottom of the desiccator in another experiment. A toxic response (reduction or absence of bacterial lawn) was found at 1.5 ml/per desiccator.

The National Toxicology Program (NTP 1983) sponsored Salmonella testing on PCE (technical grade, source Fischer) and obtained negative results. Four standard tester strains were used: TA98, TA100, TA1535, and TA1537. A pre-incubation protocol was followed in which the cells, S9 activation system, and chemical are preincubated in test tubes for 20 minutes at 37°C

^{*}Calculation based on the density of tetrachloroethylene as 1.586 g/ml.

before addition of the top agar and plating in petri plates. Two different S9 systems were used; S9 mix derived from livers of Aroclor-1254 induced rat liver and Aroclor-1254 induced hamster liver. Tests were also conducted in the absence of S9 mix. Six concentrations (0, 3, 10, 33, 100, 333 ug/plate) were evaluated. (In TA100, up to 10,000 ug/plate was evaluated.) Although incubation was carried out in capped tubes, it is possible that evaporation and some escape may have occurred. However, it should be noted that toxic levels were tested as indicated by absence or reduction of bacterial lawn.

Bartsch et al. (1979) investigated the mutagenicity of PCE (99.7 percent purity, Merck-Darmstadt) using the standard Ames/Salmonella plate test in which precautions to prevent escape of test material are not taken. Negative results were obtained using tester strain TA100 in the presence of mouse liver microsomes with and without cofactors. The authors indicated that toxic concentrations (above 82.9 mg/plate) were tested, but did not indicate their criteria for determining toxicity. Interpretation of these negative conclusions is limited by the amount of data presented and because only one tester strain was evaluated.

In an abstract, Cerna and Kypenova (1977) reported that in a spot test protocol of the Ames/Salmonella assay, PCE (purity and source not reported) induced both base-pair substitution and frameshift mutations in Salmonella typhimurium. The results were obtained in the absence of exogenous activation. These authors also reported that in a host-mediated assay using female ICR mice, PCE induced significant increases in the number of revertants in tester strains TA1950, TA1951, and TA1952 at dosage levels reported as representing the LD₅₀ and one-half of the LD₅₀. These results were reported as not being dose-dependent. Because this report was in abstract form and did not provide details of the protocol nor present the data, the acceptability of the test results is indeterminate. Also, the possibility of mutagenic contaminants must be considered.

Beliles et al. (1980) used a host-mediated assay to evaluate the effects of PCE (91.43 percent pure from North Strong Division Chemicals) in the presence of whole mammal metabolism. In this study, Salmonella tester strain TA98 was used as the indicator organism, and male and female mice (strain CD-1) were the hosts. The animals were exposed by inhalation 7 hours per day for 5 days to either 100 ppm or 500 ppm. Bacteria were injected intraperitoneally into the mice after the last exposure. The bacteria were removed

from the mice 3 hours later. At the 100 ppm dose level, increases in the number of revertants were observed for males (approximately twofold increase) but not for females. At 500 ppm, a positive response was reported for females (approximately fourfold increase) but not for males. Because of the lack of a dose-response and the weak responses, these findings should be viewed with caution. Also, the material used was of low-purity, and the responses may have been due to contaminants and/or added stabilizers. In addition, parallel *in vitro* plate tests using Salmonella were not conducted. The parallel plate tests are important the determination of the requirement of whole-mammal activation.

Studies on PCE have also been conducted in Escherichia coli. Henschler (1977) reported that tetrachloroethylene was not mutagenic when tested in E. coli K12 with metabolic activation (liver microsomal fraction prepared from phenobarbital-induced mice). This report, however, is difficult to evaluate because actual revertant count data (experimental and control number of revertants) and the details of the protocol are not provided. It appears that the conclusions presented in this report are actually based on data derived from the study by Greim et al. (1975).

Greim et al. (1975) reported that negative results were obtained when PCE (purity reported as analytical grade; Merck-Darmstadt) was assayed in the multi-purpose test system of Escherichia coli K12/343/113 (). Tests were conducted in the absence and presence of metabolic activation (phenobarbital-induced mouse liver microsomal fraction plus NADPH cofactors) at a concentration of 0.9 mM and a treatment time of 2 hours in liquid suspension at 37°C. These treatment conditions resulted in 99 ± 1 percent survival. The genetic markers evaluated for mutation induction were the missense marker arg^+ and the frameshift marker nad^+ for reverse mutation, and gal^+ and MTR for forward mutation. Deficiencies in the study design and reporting of the results reduce the weight of the negative conclusion. These deficiencies are as follows: 1) only one concentration was evaluated, 2) adequate exposure may not have been achieved, as indicated by the high survival, 3) there was no reporting of revertant count data (experimental and control), and 4) there was no reporting of the number of replicate plates used or the number of the tests conducted.

The bacterial tests discussed above do not clearly demonstrate that PCE itself is mutagenic. The positive responses found may be due to contaminants and/or added stabilizers. The induced increases in revertants did not require

exogenous metabolic activation and were observed in both frameshift and base-pair substitution-sensitive tester strains. The positive findings were not considered strong in that large amounts of material (estimated vapors at 2.5 percent v/v and at 160 mg/plate) were needed for the detection of mutagenicity. Also, there was a very narrow range of effective concentrations because of toxicity; thus dose-response relationships were not established. When tested, highly purified samples were not detected as mutagenic under the conditions in which technical samples caused increases in the number of revertants. Although some technical tetrachloroethylene samples were positive, there were other samples that were not detected as positive. The available results provide suggestive evidence that certain technical samples of PCE are weakly mutagenic in Salmonella and that the positive responses may be due to impurities and/or added stabilizers.

8.1.2 Drosophila

Beliles et al. (1980) used the sex-linked recessive lethal assay in Drosophila melanogaster to test PCE (91.43 percent purity, North Strong Division Chemicals) and reported negative results. Adult male flies were exposed for 7 hours by inhalation at 100 ppm and 500 ppm. Treated males were mated to nontreated females at various times (2-3-3-2 day mating scheme) to test specific germ cell stages. No significant increases ($P < 0.05$) over the background values were observed. However, only a small sample size was examined. A total of 3804 chromosomes for the 100 ppm dose and 3956 chromosomes for the 500 ppm dose were evaluated. This sample size was only large enough to exclude the induction of an approximately fourfold increase in mutation frequency (Kastenbaum and Bowman 1970). Ideally, at least 7000 chromosomes at each dose level should be screened to preclude a doubling in mutation frequency, which is generally considered to be the increase of biological significance. Survival was not reported in this study, and thus it is uncertain whether a sufficient dose was given. These deficiencies prevent a judgment regarding the mutagenic activity of PCE in Drosophila.

The ability of PCE to cause gene mutations in an eucaryotic organism has not been adequately examined. The only available study was a sex-linked recessive lethal test in Drosophila in which PCE was not properly evaluated.

8.2 CHROMOSOMAL ABERRATION TESTS

8.2.1 Whole-Mammal Bone Marrow Cells

Rampy et al. (1978) examined bone marrow cells for chromosome aberrations from male and female Sprague-Dawley rats after PCE exposure. Animals were exposed to 300 ppm (2.03 mg/l) or 600 ppm (4.07 mg/l) PCE* by inhalation 6 hr/day, 5 days/week, for one year. Three animals per dose were examined. The authors reported "zero" chromosomal aberrations per cell for both females and males. The data for females, however, are inadequate for a clear interpretation because of the very low number of metaphases scored (less than 25 cells per animal). In male rats, 150 cells were scored (50 cells per animal). The negative controls were also reported as "zero" aberrations. This observation is very unusual because, in general, most laboratories have reported about 1 to 2 percent total aberrations for background values. In this study, it is not known whether the highest exposure level was near the maximum tolerated dose (MTD) for females because no weight loss and no mortality was observed. In males there was no weight loss, but significant increases in mortality above control values were observed at the highest dose tested, and therefore an MTD may have been approached. It is not apparent that the investigators determined the toxicity of the test material to arrive at an MTD for this study, because the dosage levels used were based on the threshold limit value of 100 ppm for PCE.

A rat bone marrow assay was also performed by Beliles et al. (1980) and reported as negative. Ten males and ten females [CRL:COBS CD(SD)BR] were exposed to an acute dose of 100 ppm and 500 ppm PCE (91.43 percent purity, North Strong Division Chemicals) by inhalation for 7 hours. Bone marrow cells were harvested 6, 24, and 48 hours later. No increase in aberrations was found for females, but for males, weak clastogenic effects (breaks, fragments, deletions, and aneuploid cells) were observed. At 500 ppm, 3.3 percent cells with aberrations versus 0.7 percent in the control were found at the 24-hour kill. A subchronic study (five exposures, 7 hours per day) at 100 ppm and 500 ppm was also conducted. Animals were killed 6 hours after the last exposure. No increase in abnormalities was found in males, and only a slight increase at 100 ppm (1 percent cells with aberrations) was found in females. This response

*Formulation (liquid volume percent): trichloroethylene, 3 ppm; hexachloroethane, <12 ppm; carbon tetrachloride, 2 ppm; 4-methyl morpholine, 44 ppm; nonvolatile residue, 2 ppm; and tetrachloroethylene balance.

was not dose-related. The female subchronic control group had a very low background (0.3 percent cells with aberrations). Although isolated incidences of increases in chromosomal aberrations were observed, the lack of dose-responses precludes an unequivocal positive conclusion. On the other hand, a negative conclusion cannot be drawn because the authors did not discuss the criteria used to select the dosage levels, and thus, there is the possibility that a toxic dose may not have been evaluated.

Cerna and Kypenova (1977) reported in an abstract that mice (ICR) given an acute intraperitoneal dose one half of the LD₅₀ of PCE or dosed intraperitoneally for five applications in 24-hour intervals (dose of one injection equalled 1/6 LD₅₀) did not show cytogenetic effects in the bone marrow cells. Details of the protocol and the cytogenetic data were not available for an evaluation. Hence, the negative conclusion of the authors cannot be considered definitive.

8.2.2 Human Peripheral Lymphocytes

Ikeda et al. (1980) studied chromosomal aberrations, sister chromatid exchanges (SCEs), and variation in the mitotic index of peripheral lymphocytes cultured from ten workers (seven males and three females) occupationally exposed to technical grade PCE (impurities not reported). The workers were divided into high (Group 1) and low (Group 2) exposure groups. Group 1 consisted of six workers (five males and one female aged 20 to 66 years) from a degreasing workshop. These workers had a geometric mean exposure of 92 ppm (range 30 to 220 ppm). The five males of Group 1 had work histories of 10 to 18 years, whereas the one female had worked in the degreasing shop for only 1 year. Group 2 included four workers (two males and two females aged 17 to 31 years) from a support department with a shorter work history (3 months to 3 years) and with an exposure range of 10 to 40 ppm. The control group consisted of six males and five females. The authors did not indicate if this was a matched control, and did not indicate the medical histories of the subjects (e.g., recent illnesses, radiation exposures). There were no statistically significant differences ($P > 0.05$) in the incidences of chromosomal aberrations (structural and numerical) and SCEs between the exposed workers and control group. The mitotic index was similar for both exposed and control groups.

8.2.3 Drosophila

In addition to performing the sex-linked recessive lethal assay discussed earlier, Beliles et al. (1980) also conducted a sex chromosome loss assay on low-purity (91.4 percent, North Strong Division Chemicals) PCE for nondisjunction in Drosophila melanogaster. Males were exposed to 100 and 500 ppm of tetrachloroethylene for 7 hours. The phenotypic classes used allow for detection of losses of the entire X or Y chromosome and the short or long arm of the Y chromosome. Although marginal increases, which were not dose-related, were observed after PCE treatment, they are not considered sufficient to judge the data as positive or negative.

The cytogenetic tests discussed above using mice, rats, Drosophila, and exposed humans have been reported as negative. Although these studies are not considered to be a thorough evaluation of the ability of PCE to cause chromosomal aberrations, the data collectively indicates that PCE is not strongly clastogenic. However, there have been no adequate studies on the ability of PCE to cause chromosome nondisjunction (aneuploidy).

8.3 OTHER TESTS INDICATIVE OF DNA DAMAGE

8.3.1 DNA Repair

Unscheduled DNA synthesis (UDS) is measured by repair of DNA lesions, which is indicative of DNA damage. Beliles et al. (1980) assessed the ability of PCE (91.43 percent purity, North Strong Division Chemicals) to cause UDS in human fibroblast (WI-38) cells. Because WI-38 cells have little if any enzyme activation capability, the tests were conducted with an exogenous source of metabolic activation (i.e., S9 mix). The test material was examined at 0.01, 0.05, 0.1, and 0.5 percent (v/v) using conventional liquid phase exposure. Scheduled DNA synthesis was blocked by treatment with hydroxyurea, and UDS was measured by liquid scintillation counting of incorporated tritiated thymidine ($[^3\text{H}]\text{-TdR}$) into DNA. A very slight increase was seen at 0.01 percent (v/v) PCE both in the presence (1.5-fold of control) and absence (1.35-fold of control) of Aroclor rat liver S9 mix. No increases occurred at 0.1 and 0.5 percent. A toxic response was reported at 0.5 percent (i.e., a decrease in total amount of DNA as well as in the incorporation of $[^3\text{H}]\text{-TdR}$). An increase in total amount of DNA at 0.01 percent was found, suggesting that more cells are entering the S phase. Cells accelerated into S phase would account for the increases seen in $[^3\text{H}]\text{-TdR}$ incorporation at 0.01 percent. Therefore, it

is uncertain whether PCE induced a weak UDS response in this system. Also, other problems were found in this study. The positive controls gave weak responses. N-methyl-N'-nitro-N-nitrosoguanidine elicited a weak response (1.8-fold increase), but at a toxic concentration (5 ug/ml) as indicated by a decrease in total DNA (6.27 ug of DNA versus 23.76 ug of DNA in solvent control). Benzo[a]pyrene gave a response of 1.16-fold above background. This is an equivocal response, particularly because there was also an increase found in the total amount of DNA.

Williams and Shimada (1983, sponsored by PPG Industries, Inc.) evaluated two different samples of PCE, Perchlor 200 (low-stabilized, 99.93 percent purity) and Perchlor 230 (stabilized, purity 99.80 percent), for their ability to cause UDS in the hepatocyte primary culture (HPC)/DNA repair test. The target cells in this test system have a capability to metabolize xenobiotics. Williams and Shimada measured UDS by autoradiographic determination of the amount of [³H]-TdR (10 uCi/ml) incorporated into nuclear DNA. Hepatocytes were isolated from adult male Fischer 344 rats. Cells were treated for 18 hours or 3 hours. For the 3-hour exposure, cultures were incubated another 15 hours in the absence of PCE to allow for DNA repair synthesis. The criterion that was used for a positive result was a net nuclear grain count of 5 in triplicate coverslips.* Negative results were reported for both Perchlor 230 (at concentrations of 0.001, 0.01, 0.1, and 1.0 percent v/v) and Perchlor 200 (at concentrations of 0.0001, 0.001, 0.1, and 1.0 percent) when tests were conducted using conventional liquid-phase exposure in which PCE was added to the culture medium.

For both materials, positive responses were reported when testing was performed using vapor-phase exposure in gas tight chambers. Testing was conducted at 0.1, 1.0, and 2.5 percent v/v (desired air concentration) for 3 and 18 hours. Perchlor 230 at 0.1 percent caused an increase in UDS when the cells were exposed for 3 hours (6.2 ± 4.9 net nuclear grain count, 50 percent cells showing toxic effects) and for 18 hours (15.9 ± 1.6 net nuclear grain

*Nuclear grain counts were reported as the mean $\pm$ standard deviation. Cytoplasmic grain counts in three nuclear size areas adjacent to the nucleus were determined. The highest cytoplasmic grain count was subtracted from the nuclear count. This value is referred to as "net" grain count.

count, 25 percent cells showing toxic effects). Perchlor 200 caused an increase in UDS at 0.1 percent v/v for the 3-hour treatment (10.8 ± 6.1 percent net nuclear grain count, 75 percent cells showing toxic effects) but not for the 18-hour treatment. At 1.0 percent and 2.5 percent for both materials at both treatment times, nearly 100 percent of cells showed toxic effects. It should be pointed out that the background control values were taken from the conventional exposure experiment. This makes interpretation of the results difficult, particularly because the conventional-phase media was different from the gas-phase media. Because these data are based on one test in which there were no concurrent positive and negative controls, the results are considered only suggestive of a positive effect. To validate these findings it is necessary to repeat experiments using appropriate concurrent controls and a concentration range to demonstrate a dose-response. Although the possibility exists that an impurity(ies) may be responsible for the observed effects, the authors did not examine high-purity Perchlor (99.98+ percent) as they did in the Salmonella tests discussed earlier. If high-purity material had tested negative under the same experimental conditions under which the lower-purity materials tested positive, as in the Salmonella test, a stronger argument could be made that impurities were causing the effects.

Williams (1983) conducted HPC/DNA repair tests using hepatocytes from male $B_6C_3F_1$ mice and male Osborne-Mendel rats on PCE (99+ percent purity, Aldrich Chemical Company). Conventional liquid-phase exposure conditions were used. Negative results were reported for both rat and mice hepatocytes when PCE was added to the culture medium at 0.00001, 0.0001, 0.001, 0.01, and 0.1 percent (v/v) for 18 hours. The material was reported as "toxic" at 0.01 percent and higher. The highest cytoplasmic grain count was subtracted from the nuclear count. This reduces the possibility of false positives, but the chance of missing a weak UDS inducer would be increased, especially if the cytoplasmic grain count is high. This consideration also applies to the Williams and Shimada conventional-phase exposure study discussed previously. The cytoplasmic grain counts were not reported in any of the HPC/DNA repair studies.

Toxicity identified by the absence of S phase cells and general cellular morphology. This is not an accurate method of determining toxicity.

8.3.2 Mitotic Recombination

Callen et al. (1980) evaluated the ability of PCE (purity not reported, stabilized 0.01 percent thymol, Eastman Kodak) to cause mitotic gene conversion (nonreciprocal recombination) at the *trp-5* locus, mitotic crossing-over (reciprocal recombination) at the *ade-2* locus, and gene mutation (reversion) at the *ilv-1* locus in log phase cultures of *Saccharomyces cerevisiae* D7. Cells were incubated for one hour in culture medium containing 0, 4.9, 6.6, and 8.2 mM PCE. No exogenous source of metabolic activation was used in these studies. At 4.9 mM (84 percent survival), no significant increases in the frequency of gene conversion (1.9 convertants/ 10^5 survivors versus 1.4 convertants/ 10^5 survivors in background control) and mitotic crossing-over (5.3 mitotic recombinants/ 10^4 survivors versus 3.3 mitotic recombinants/ 10^4 survivors in background control) occurred. As shown in Figure 8-2, when the concentration of PCE was increased to 6.6 mM (58 percent survival), increases in mitotic gene conversion and mitotic crossing-over did occur (8.3 convertants/ 10^5 survivors and 52.6 mitotic recombinants/ 10^4 survivors, respectively). Mitotic recombinational activity was not determined at 8.2 mM because less than 0.1 percent survival was found. No significant increases in gene mutations were observed at 4.9 mM (3.8 revertants/ 10^6 survivors versus 2.9 revertants/ 10^6 survivors in control). The reverse mutation frequency was not determined at 6.6 mM. Therefore, the induced number of revertants is too low to be indicative of a positive result, but the high values for the recombinational events do indicate a positive effect at 4.9 mM test material. The possibility that the effects were caused by a mutagenic impurity(ies) should be considered. It should be pointed out that in the study by Callen et al. the Ade^+ recombinants were estimated from a total of 30 plates, ten of which contained minimum medium (plus adenine and isoleucine) used for estimating the number of *trp-5* convertants. Because mitotic crossing-over and gene conversion are not necessarily distinct events, in that they probably depend on a common inducible mechanism, the number of Ade^+ recombinants may have been overestimated by including those Ade^+ that were already Trp^+ . There is experimental support that these recombinational events are inducible by a common factor(s) (see Fabre Fabre and Roman 1977, Fabre 1978). The positive findings of Callen and coworkers for mitotic recombination should be confirmed by repeating the assay using appropriate selection conditions. In addition, because the responses were observed within a narrow "window," at least one additional concentration between the low

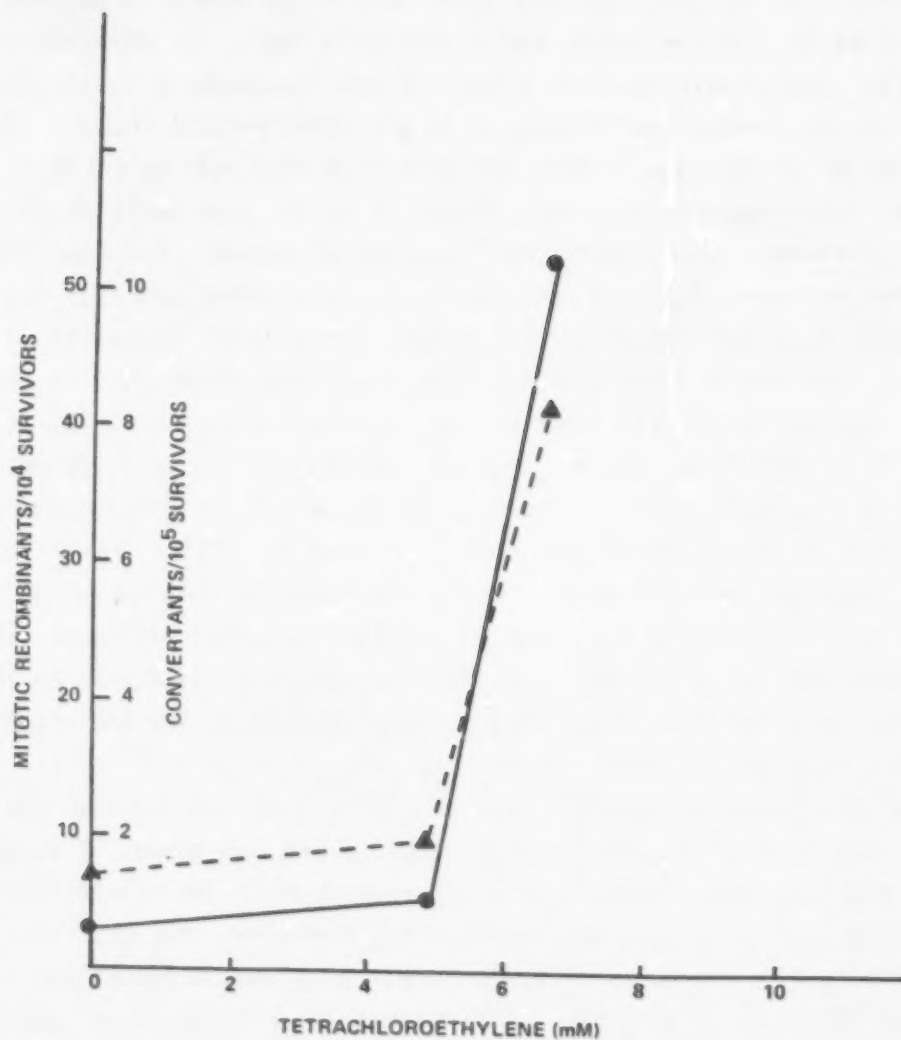


Figure 8-2. Induction of mitotic recombination by tetrachloroethylene in *Saccharomyces cerevisiae* D7. The frequency of mitotic crossing-over (●—●) and gene conversion (▲---▲) was determined. Log-phase yeast cells were treated with test chemical for one hour without the addition of an exogenous metabolic activation system. (Adapted from Callen et al. 1980)

least one additional concentration between the low and high doses used should be tested to demonstrate a clear dose-response.

Bronzetti et al. (1983) also used the yeast S. cerevisiae D7 to evaluate the effects of PCE (99.5 percent pure, stabilized with 0.01 percent thymol, Carlo Erba Co.) at the *trp-5*, *ade-2*, and *ilv-1* loci. In this study, however, negative results were obtained after a 2-hour treatment at 5, 10, 20, 60, and 85 mM in the presence or absence of S9 mix (Aroclor-1254 induced rat liver). Differences in the experimental protocol from that used by Callen et al. may explain these negative findings. Bronzetti et al. used cells in the stationary phase of growth rather than in the log phase of growth. For some chemicals it has been observed that stationary cells are more refractory than log cells to mutagenic treatment (Mayer and Goin 1980, Shahin 1975). Bronzetti et al. were able to test higher concentrations than Callen and coworkers. In the absence of S9 mix, Callen et al. reported less than 0.1 percent survival at 8.2 mM, while Bronzetti et al. did not observe complete killing until 85 mM. Another difference between these two studies is the source of tetrachloroethylene. Bronzetti et al. purchased PCE from Carlo Erba Co. (Milan, Italy) and Callen et al. obtained the test agent from Eastman Kodak Co. (Rochester, N.Y.); thus, it is possible that the test samples may have contained different impurities.

Bronzetti et al. (1983) also obtained negative results in an intrasanguineous host-mediated assay using S. cerevisiae D7 as the indicator organism and CD-1 mice as the host. Stationary yeast cells (4×10^8 cells) were injected into the retro-orbital sinus of mice. After injection of the yeast, an acute oral dose of 11 g PCE/kg body weight (b.w.) was given. A subacute dose of 2 g/kg b.w. given 5 days a week for a total of 12 administrations was also used (the last test dose was 4 g/kg b.w.; therefore, the total dose was 26 g/kg b.w.). In the subacute study, yeast cells were injected after the last dose of PCE (i.e., 4 g/kg b.w.). Four hours after injection of yeast, in both the acute and subchronic study, the cells were recovered from the liver, lungs, and kidneys of three animals. No concurrent positive control chemical was used in these studies to ensure that the system was functioning properly.

The positive responses discussed above for mitotic recombination in yeast and DNA repair synthesis in mammalian cells provide suggestive evidence that certain technical samples of PCE may be active in damaging DNA. However, toxic concentrations of material were needed to elicit these responses. The possibility that impurities and/or added stabilizers caused the increased effects should be considered.

8.4 DNA BINDING STUDIES

Chemical adduct formation is a critical step in certain types of mutagenesis. Schumann et al. (1980) reported that there was no detectable binding of ^{14}C -labeled PCE (99 percent purity) to DNA when mice were treated by inhalation at 600 ppm for 6 hours or when they were given an acute dose of 500 mg/kg orally. The specific activity of the ^{14}C -label (26,593 dpm/umol in the inhalation study and 133,273 dpm/umol in the oral study) is too low, however, to preclude the possibility of very low levels of DNA binding. For example, at the specific activities used under the experimental conditions, at an assumed binding level of 10^{-5} alkylations per nucleotide (Stott and Watanabe 1982) there would be 5-10 dpm per sample. This is at the limit of practical detection. Therefore, the possibility of binding at slightly less than 10^{-5} alkylations per nucleotide cannot be ruled out. These negative findings, however, are consistent with the negative and weak results reported in the mutagenicity tests discussed above.

8.5 STUDIES INDICATIVE OF MUTAGENICITY IN GERM CELLS

An important aspect of a mutagenicity evaluation is to assess the potential of the chemical to reach the germinal tissue of humans and cause mutations that may contribute to the genetic disease burden. This assessment is almost always based on animal experimentation. The ability of PCE to cause genetic damage in germinal tissue has not been well studied. The only test results available were from a dominant lethal study in rats [CRL: COBS CD(SD)BR] and a sperm morphology assay in both rats and mice (strain CD-1). Tetrachloroethylene (91.43 percent purity, North Strong Division Chemicals) did not cause an increase in dominant lethals in rats when unexposed females were mated during a 7-week period to exposed adult males given an acute dose of 100 ppm and 500 ppm by inhalation for 7 hours per day for 5 days (Beliles et al. 1980). The dominant lethal assay is generally thought to measure gross chromosome damage (Bateman and Epstein 1971). Also, this test is not considered a sensitive assay because of the high spontaneous level of lethal events (Russell and Matter 1980), and thus, negative results do not necessarily indicate that the chemical does not reach and damage the germ cell DNA.

Beliles et al. (1980) also examined PCE for altered sperm morphology in treated rats and mice. After dosing at 100 ppm and 500 ppm by inhalation 7 hours per day for 5 consecutive days, groups of four animals were killed at

the end of 1 week, 4 weeks, and 10 weeks to examine effects on various germ cell stages. Sperm was collected from the cauda epididymis, and at least 500 cells were examined. Negative results were reported in rats; the mice, however, showed positive responses. At 500 ppm, 19.7 percent abnormal sperm were observed (versus 6.0 percent in the negative controls) during the fourth week after exposure (corresponding to the spermatocyte stage). By themselves, these positive findings alone are not sufficient to conclude that PCE alters germ cell DNA because this assay is only an indicator of chemical effects on sperm and does not provide definitive evidence that a chemical reached germinal tissue and damaged DNA. Therefore, because limited information was provided, it is not clear whether PCE (or impurities) reaches the germ tissue. However, if PCE reached germ cell DNA, there may be no serious risk of mutation because of the largely negative or marginal results found in mutagenicity tests discussed previously.

8.6 MUTAGENICITY OF METABOLITES

Trichloroacetic acid (TCA) is a known human metabolite of PCE. The formation of TCA is thought to occur through the formation of an epoxide, tetrachloroethylene oxide, and its subsequent rearrangement to trichloroacetyl chloride or trichloroacetaldehyde, which then rapidly hydrolyzes to TCA. (See chapter 5 on metabolism for a more detailed discussion.) These intermediates are considered relevant in assessing the mutagenicity of PCE.

Tetrachloroethylene oxide, which is considered to be the biologically active intermediate of the parent compound PCE, was assayed for mutagenicity in the absence of exogenous metabolic activation in several bacterial tests (Kline et al. 1982). Tetrachloroethylene oxide increased the number of revertants in a dose-dependent manner in Salmonella tester strain TA1535 when assayed by a preincubation liquid protocol (20 minutes at 37°C), but did not cause an increase in revertants using Escherichia coli WP2 uvr A. In Salmonella, a 14-fold increase occurred at 2.5 mM and a 20-fold increase occurred at 5mM. Tetrachloroethylene epoxide was toxic at 25mM.

This epoxide was also evaluated by Kline et al. (1982) in the E. coli pol A assay. Positive effects were observed at 0.04, 0.09, and 0.44 mM/ml as measured by differential growth inhibition of a DNA polymerase-deficient strain in comparison with its polymerase-proficient parent.

Waskell (1978) tested TCA at 0.45 mg/plate in Salmonella TA98 and TA100 and obtained negative results. There are other intermediates which have not been identified in humans but are thought to occur (e.g., trichloroethanol chloral hydrate). Waskell (1978) obtained negative results for trichloroethanol in Salmonella TA98 and TA100 up to a dose of 7.5 mg/plate. Gu et al. (1981), however, reported suggestive evidence that trichloroethanol weakly induced sister chromatid exchange (SCE) formation in primary cultures of human lymphocytes.

Chloral hydrate was reported to be marginally mutagenic (less than twofold increase over a dose range of 0.5 to 10 mg per plate) in Salmonella TA100 (Waskell 1978). Gu et al. (1981) also provided suggestive evidence that chloral hydrate at 54.1 mg/l caused a weak increase in SCEs in cultured human lymphocytes. Chloral hydrate has also been shown to block spindle elongation in insect spermatocytes (Ris 1949). Data on metabolites of PCE suggest that if the parent compound was biotransformed, its metabolites may be genotoxic; these data are limited, however, and additional studies are needed on metabolism and on the mutagenicity of metabolites to reach a clear conclusion.

8.7 SUMMARY AND CONCLUSIONS

Tetrachloroethylene itself has not been clearly shown to be a mutagen. Certain commercial and technical preparations have elicited positive responses in the Ames bacterial test, a yeast recombinogenic assay, a host-mediated assay using Salmonella, and DNA repair assays. In general, the responses were weak, and eliciting them required rather high toxic concentrations of tetrachloroethylene. No dose-response relationships were established in these studies. The positive findings may be explained by the presence of mutagenic contaminants and/or added stabilizers. Highly purified tetrachloroethylene has only been evaluated in the Ames/Salmonella test, where negative results were obtained.

Several other tests of commercial and technical samples have been reported to be negative. In addition, The National Toxicology Program (NTP) has recently sponsored mutagenicity testing (a modified Ames Salmonella test, a sex-linked recessive lethal test in Drosophila, sister chromatid exchange formation and chromosome aberrations in Chinese hamster ovary cells in vitro on a technical sample of PCE; the preliminary results were negative. (These studies are in the process of being peer-reviewed, and were not discussed in this chapter,

except for the Ames test.) The inconsistencies of available results on different samples of PCE may be a function of the toxicity of the test material, of exposure conditions used for testing this volatile chemical, or of differences in sample contaminants and/or added stabilizers. Information on chemical composition of the PCE test samples was scarce.

Although PCE itself has not been shown to be mutagenic, it should be emphasized that the negative results are not wholly unequivocal. Appropriate concurrent controls, adequate sample sizes, and exposure conditions were sometimes not used, and in some cases the available data are not sufficient to determine whether an adequate test was conducted. Also, there have been no reliable studies investigating the ability of PCE to cause chromosome nondisjunction, which would result in aneuploidy, a significant genotoxic effect.

Because the epoxide of PCE was mutagenic in bacterial studies, the concern should be raised that it may pose a mutagenic hazard. It should be noted, however, that the parent compound was assayed in the presence of several types of metabolic activation systems (i.e., liver homogenates, intact hepatocytes, and whole mammals) and the results were largely negative or weakly positive. Therefore, it is uncertain whether these negative or weak findings were the result of limitations of the activation systems, the epoxide not being produced in sufficient quantities, or the epoxide possessing too short a half life to cause a detectable mutagenic response.

In conclusion, inadequate information exists to warrant a provisional classification of PCE either as nonmutagenic or mutagenic. If PCE is a mutagen, the evidence available thus far indicates that it is only weakly so. (Because of insufficient information, this conclusion is not made with regard to its potential for causing chromosome nondisjunction.) Certain commercial and technical preparations of PCE may contain mutagenic impurities and/or added stabilizers. Although there may be mutagenic agents in certain preparations of PCE, usually large amounts of material (at toxic levels) were required to elicit weak responses.

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9. CARCINOGENICITY

The purpose of this section is to provide an evaluation of the likelihood that tetrachloroethylene (perchloroethylene, PCE) is a human carcinogen and, on the assumption that it is a human carcinogen, to provide a basis for estimating its public health impact, including a potency evaluation, in relation to other carcinogens. The evaluation of carcinogenicity depends heavily on animal bioassays and epidemiologic evidence. However, information on mutagenicity and metabolism, particularly in relation to interaction with DNA, as well as to pharmacokinetic behavior, has an important bearing on both the qualitative and quantitative assessment of carcinogenicity. The available information on these subjects is reviewed in other sections of this document. This section presents an evaluation of the animal bioassays, the human epidemiologic evidence, the quantitative aspects of assessment, and finally, a summary and conclusions dealing with all of the relevant aspects of the carcinogenicity of PCE.

9.1 ANIMAL STUDIES

Two long-term animal bioassays have been performed to assess the carcinogenic potential of PCE. In one study involving exposure of rats and mice to PCE by gavage, the National Cancer Institute (NCI) (1977a) reported the induction of hepatocellular carcinomas in male and female mice, but determined that the test with rats was inconclusive because of excessive mortality. In the other study, in which Sprague-Dawley rats were exposed to PCE by inhalation, the Dow Chemical Company (Rampy et al., 1978) reported no evidence for the carcinogenicity of the chemical. However, limitations in this study make it difficult to assess the carcinogenic potential of PCE.

9.1.1 National Cancer Institute Bioassay (1977a)

The PCE sample used in this bioassay was purchased from the Aldrich Chemical Company, Milwaukee, Wisconsin. Analysis by gas-liquid chromatography and infrared spectroscopy yielded results indicating a purity of > 99% with at least one minor impurity not identified in the report. Identification of the impurities in the test sample was not made (personal communications with the NCI and the Aldrich Chemical Company).

The carcinogenicity of PCE was tested in Osborne-Mendel rats and B6C3F1 mice. The initial age of the weanling animals was 25 days for the mice and 35 days for the rats. Two treatment groups consisted of 50 males and 50 females, and matched vehicle (corn oil) and untreated control groups comprised 20 animals of each sex. Selected dosage levels were those determined to be maximally tolerated in an 8-week subchronic study, i.e., a dosage that was not fatal and/or did not reduce body weight gain more than approximately 20%, and one-half maximally tolerated in an 8-week subchronic toxicity test. Time-weighted average doses (mg/kg/dose) in the chronic study were 941 and 471 for male rats, 949 and 474 for female rats, 1,072 and 536 for male mice, and 772 and 386 for female mice. PCE was administered to the animals by gastric intubation in corn oil once each day, 5 days/week, for 78 weeks. During the final 26 weeks of treatment, doses were administered to rats in a cyclic pattern of 1 week without treatment followed by 4 weeks with treatment. Body weights and food consumption were obtained weekly for the first 10 weeks and monthly thereafter. Mice and rats were permitted to survive an additional 12 and 32 weeks after treatment, respectively, until sacrifice.

Each animal was submitted to extensive gross and microscopic examinations. Specified organs, plus any other tissue containing visible lesions, were fixed in 10% buffered formalin, embedded in paraplast, and sectioned for slides.

Hematoxylin and eosin staining (H and E) was used routinely, but other stains were employed when needed. Diagnoses of observed tumors and other lesions were coded according to a modified Systematized Nomenclature of Pathology (SNOP) originally developed by the College of American Pathologists in 1965.

PCE was found to be carcinogenic in mice in this study. Results summarized in Table 9-1 indicate that PCE induced highly statistically significant ($p < 0.001$) increases in the incidence of hepatocellular carcinomas in both sexes of mice in both treatment groups as compared to untreated controls or vehicle-controls. The microscopic appearance of carcinomas was variable, with some tumors composed of well-differentiated hepatocytes arranged in rather uniform hepatic cords, and other lesions consisting of anaplastic cells, often with inclusion bodies with vacuolated, pale cytoplasm. Mitotic figures were often present. In male mice, the first hepatocellular carcinomas were detected at 27 weeks in the low-dose group, 40 weeks in the high-dose group, and 90 and 91 weeks in vehicle-control and untreated control groups. In female mice, the first hepatocellular carcinomas were observed at week 41 in the low-dose group, week 50 in the high-dose group, and week 91 in the untreated control group. Metastases of hepatocellular carcinomas occurred in the kidneys of one untreated control male and in the lung of three low-dose males, one low-dose female, and one high-dose female.

Toxic nephropathy in mice was apparent in 40/49 low-dose males, 45/48 high-dose males, 46/48 low-dose females, and 48/48 high-dose females. Control animals did not exhibit this lesion. Chronic murine pneumonia was also a frequently observed finding. A low incidence of bloating or abdominal distension was noted in treated animals during the second year of the study. Body weight gain was comparable between groups (Figure 9-1). Median survival times were greater than 90 weeks in control males, 78 weeks in low-dose males, and 43

TABLE 9-1. INCIDENCE OF HEPATOCELLULAR CARCINOMAS
IN B6C3F1 MICE FED PCE

Dose (mg/kg/day) ^a	Hepatocellular carcinomas	p values ^b
<u>Males</u>		
untreated	2/17 (12%)	
vehicle-control	2/20 (10%)	
536	32/49 (65%)	p < 0.001
1072	27/48 (56%)	p < 0.001
<u>Females</u>		
untreated	2/20 (10%)	
vehicle-control	0/20 (0%)	
386	19/48 (40%)	p < 0.001
772	19/48 (40%)	p < 0.001

^aTime-weighted average doses.

^bProbability level (p-values) for the Fisher Exact Test comparison of dose groups with vehicle-control group.

SOURCE: National Cancer Institute, 1977a.

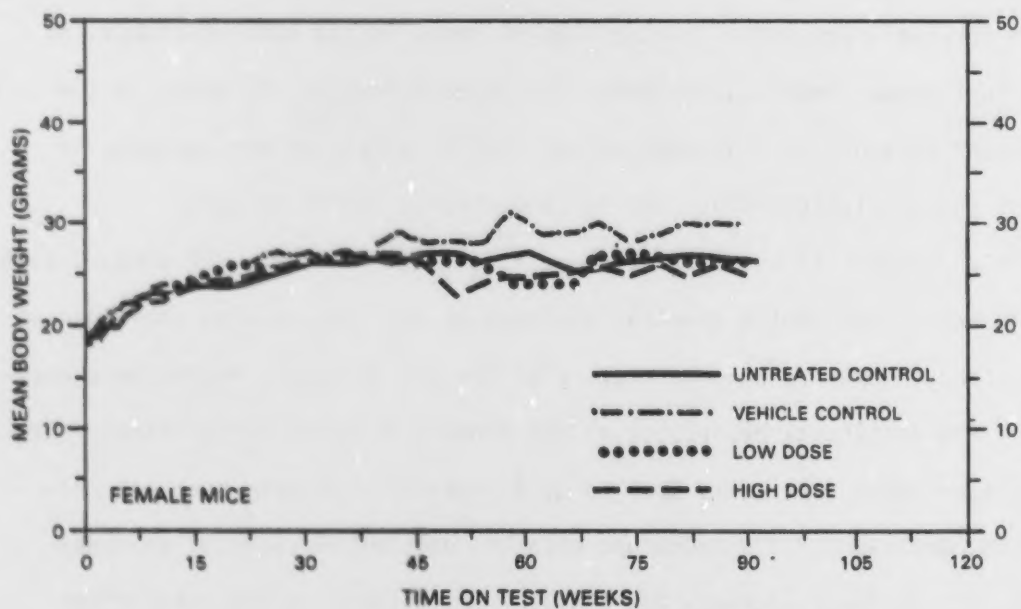
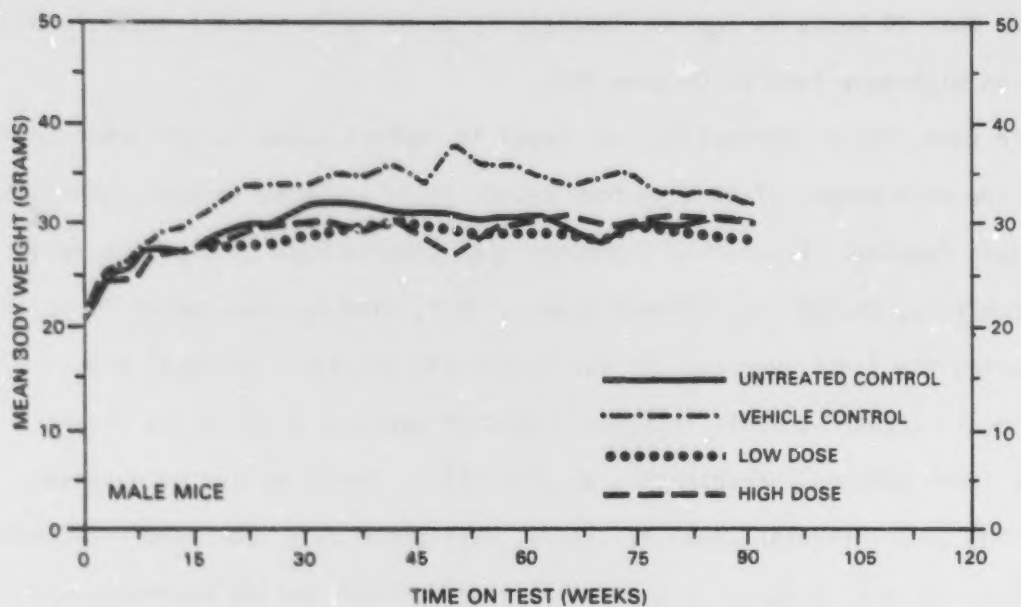


Figure 9-1. Growth curves for male and female mice in the PCE chronic study.

SOURCE: National Cancer Institute, 1977a.

weeks in high-dose males (Figure 9-2). Median survival times for females were greater than 90 weeks in control females, 62 weeks in low-dose females, and 50 weeks in high-dose females (Figure 9-2).

In rats, toxic nephropathy, not found in control animals, was detected in 43/49 low-dose males, 47/50 high-dose males, 29/50 low-dose females, and 39/50 high-dose females. Figure 9-3 indicates that treated rats gained less weight than controls, though the difference was slight, with maximum reduction being 13% during the first year and 19% during the second year. Clinical signs apparent in treated animals included a hunched appearance and urine stains on the lower abdomen. Respiratory abnormalities, characterized by dyspnea, wheezing, and/or reddish nasal discharge, were noted with increased incidence in all groups during aging of the animals, and chronic murine pneumonia was diagnosed in $\geq 62\%$ of the animals in each group. As indicated in Figure 9-4, median survival times were greater than 88 weeks in the control groups, 68 weeks in low-dose females, 66 weeks in high-dose females, 67 weeks in low-dose males, and 44 weeks in high-dose males. The survival was not adequate to support any conclusions about the carcinogenicity of PCE in rats.

In an attempt to characterize impurities present in the PCE product used in this study, documented chemical analyses of the test samples performed at the carcinogenicity testing laboratory of the NCI bioassay program were examined by the analytical department of the Diamond Shamrock Corporation (interoffice memorandum from E. A. Rowe to G. K. Hatfield, Diamond Shamrock Corporation, October 8, 1979, obtained with the documented chemical analyses from G. K. Hatfield, January 28, 1981). As indicated in the interoffice memorandum, PCE samples used in the National Cancer Institute bioassay (NCI, 1977a) were not available for analysis at Diamond Shamrock; however, the analytical method, with the same type of instrument and column used at the

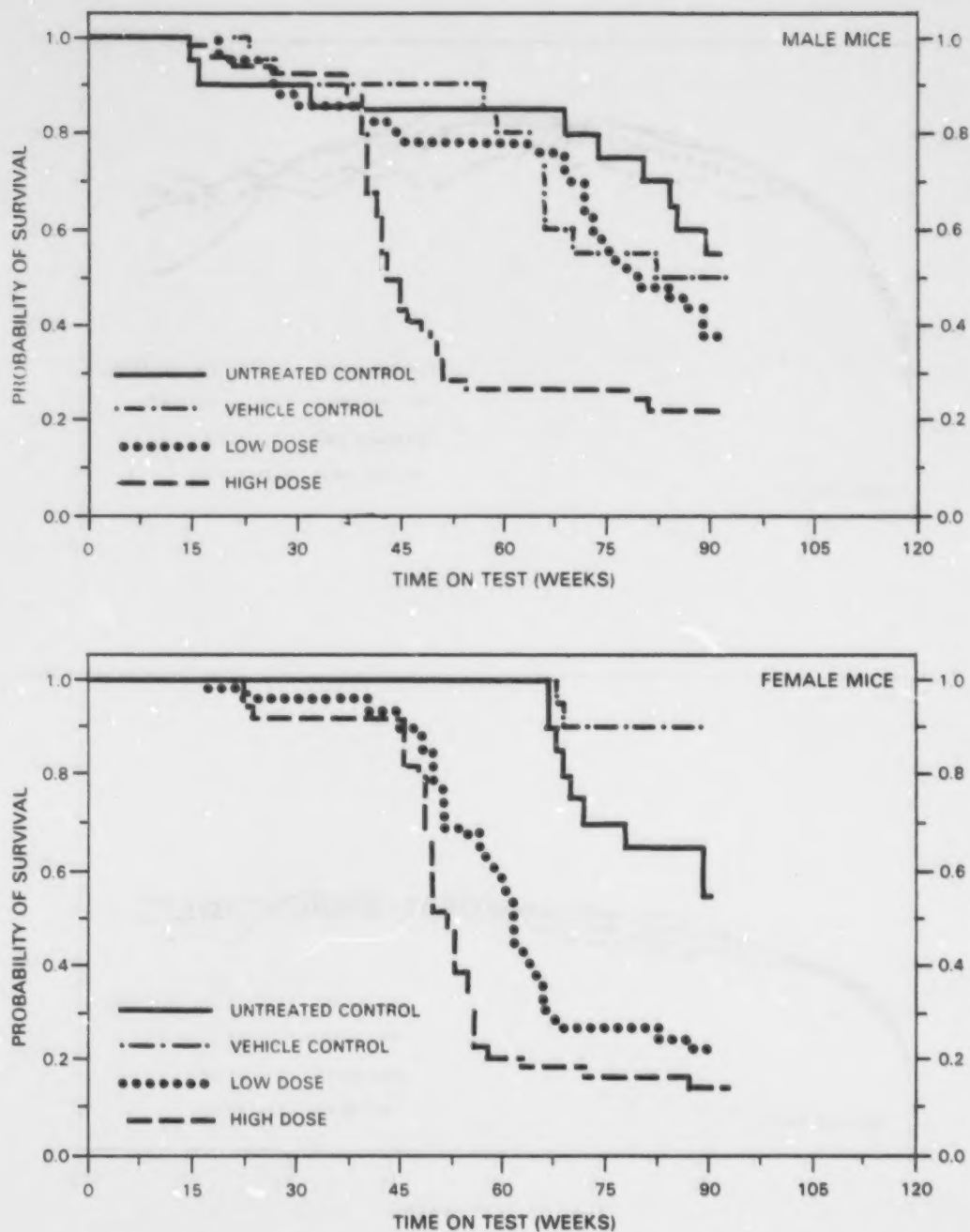


Figure 9-2. Survival comparisons of male and female mice in the PCE chronic study.

SOURCE: National Cancer Institute, 1977a.

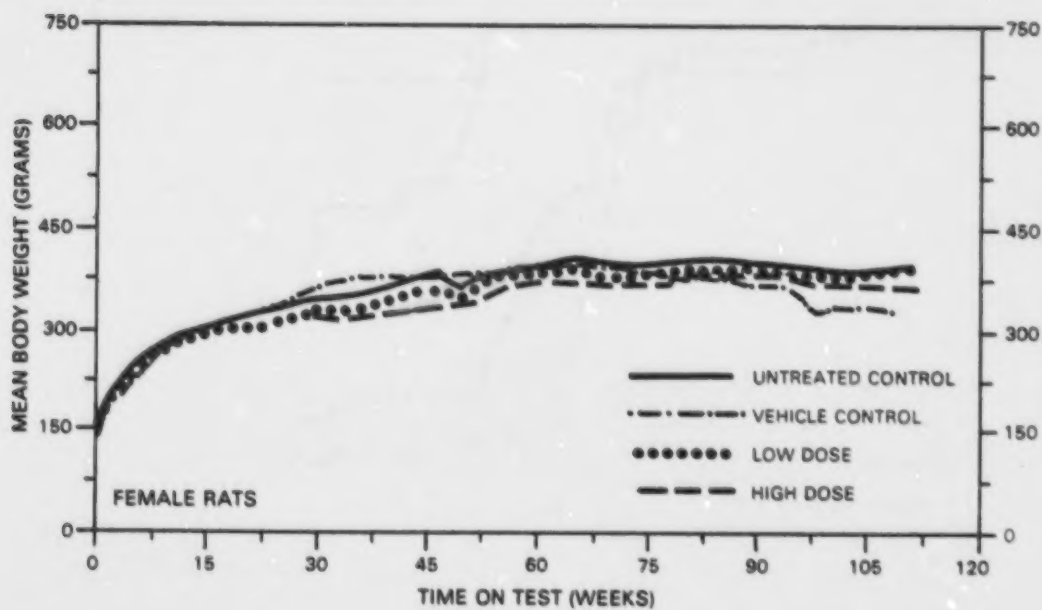
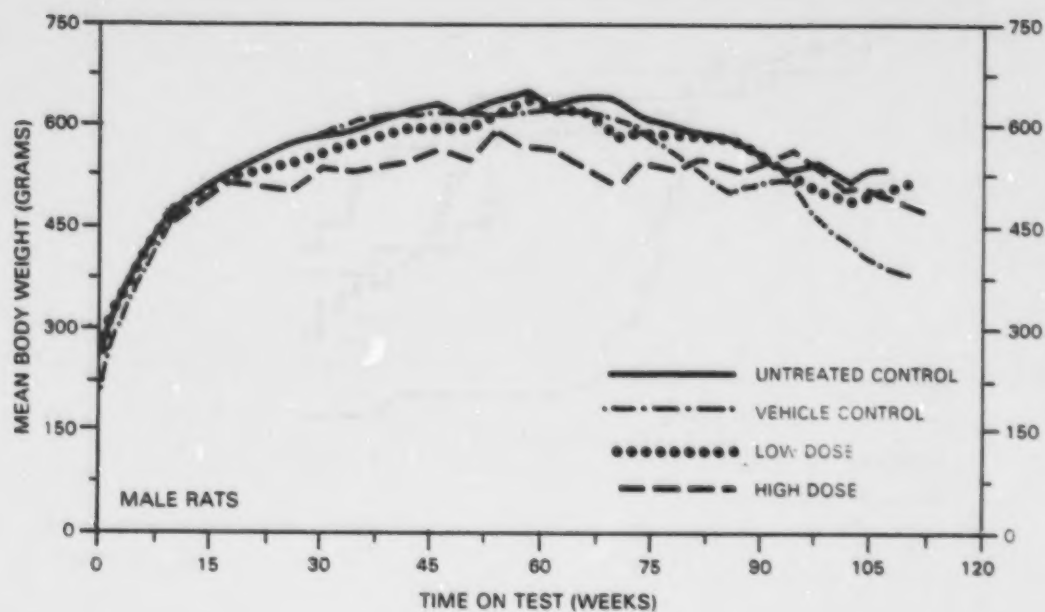


Figure 9-3. Growth curves for male and female rats in the PCE chronic study.
SOURCE: National Cancer Institute, 1977a.

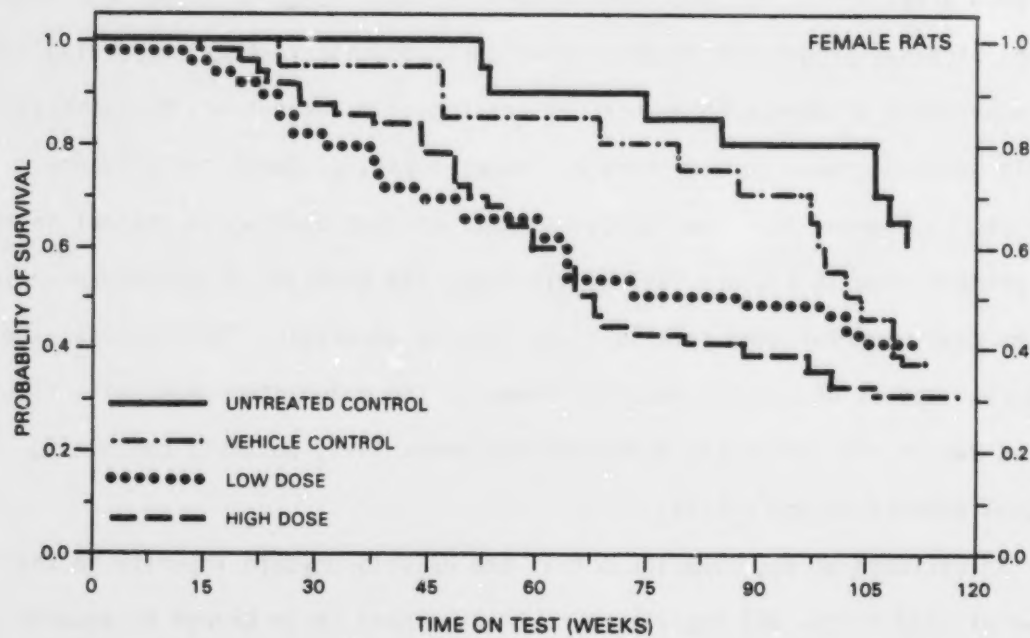
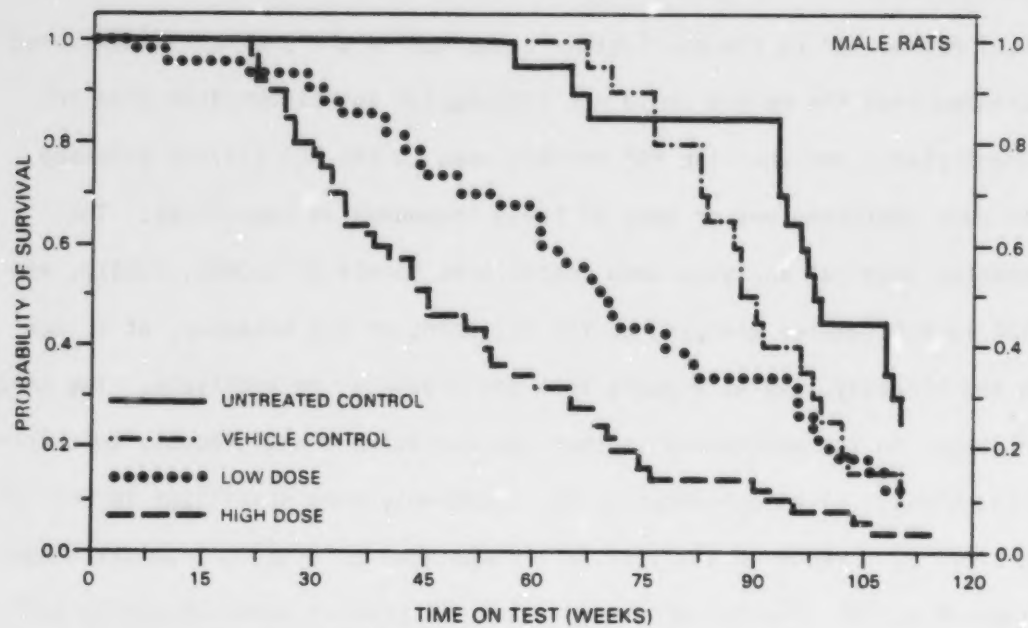


Figure 9-4. Survival comparisons of male and female rats in the PCE chronic study.

SOURCE: National Cancer Institute, 1977a.

carcinogenicity testing laboratory, was reproduced. Evaluation of the analytical method led to the conclusions presented in the previously mentioned memorandum that the method could not distinguish epichlorohydrin from trichloroethylene, and that the PCE product used in the NCI (1977a) bioassay could have contained one or both of these compounds as impurities. The documented chemical analyses show contaminant levels of 0.055%, 0.041%, and 0.010% in PCE samples analyzed at the beginning of the bioassay, at 1 year into the bioassay, and at 2 years into the bioassay, respectively. The conclusion stated in the memorandum is that the contaminant was probably epichlorohydrin, since: 1) epichlorohydrin was a commonly used stabilizer for PCE at that time; 2) a reported analysis by a competitor of Diamond Shamrock showed a maximum of 0.015% trichloroethylene in any PCE product manufactured in the United States, and 3) the decreased amount of impurity found in the PCE samples as the bioassay progressed suggests some decomposition in that, according to the experience of Diamond Shamrock, epichlorohydrin but not trichloroethylene levels would decrease during storage. Nonetheless, although the evidence provided by Diamond Shamrock indicates that epichlorohydrin was present in the PCE product used in the NCI (1977a) bioassay, the quantity of epichlorohydrin in the test material used in this study remains uncertain. Furthermore, the different levels of unknown material shown in the documented chemical analysis may be due to the use of the indicated different lots, possibly containing unequal amounts of impurities.

An estimate of the likelihood that the epichlorohydrin impurity in the PCE material used in the NCI experiment could have been large enough to account for the positive results can be obtained by considering the results of Laskin et al. (1980), who exposed rats to epichlorohydrin via inhalation. They found that 1/100 rats exposed to 30 ppm (0.115 mg/L air, equivalent to 13.2 mg/kg/day) of

epichlorohydrin for 6 hours/day, 5 days/week, for 730 days developed squamous cell nasal carcinomas, as compared with 0/50 in control animals.

The epichlorohydrin impurity in the NCI PCE high-dose male mice experiment was approximately $1,032 \text{ mg/kg/day} \times 0.041\% = 0.42 \text{ mg/kg/day}$, from the discussion above. This is only about $0.42/13.2 \approx 0.03$ times the dose that gave an incidence of only 1/100 in the Laskin et al. (1980) rat experiment. Therefore, it is unlikely that epichlorohydrin impurities at the level estimated to be present in the NCI experiment could have contributed appreciably to the positive response.

In a second carcinogenicity bioassay sponsored by the National Toxicology Program (NTP, 1983, draft), PCE was given orally to B6C3F1 mice and to four strains of rats (Sherman, Fischer 344, Long-Evans, and Wistar). The PCE sample used in this ongoing carcinogenicity bioassay did not contain detectable amounts of epoxide contaminants. In the study with female B6C3F1 mice (NTP, 1983, draft), groups of 100 females received 25, 50, 100, or 200 mg of more than 99% pure PCE per kg body weight in corn oil by gavage for 103 weeks, 5 days/week. Vehicle-control and untreated control groups of 100 mice each were used. Survival was not affected significantly. Doses of 50 mg/kg or more produced a dose-related cytomegaly of the kidneys. Other signs of liver toxicity appeared during the study as increases of sorbitol dehydrogenase activity, and increases in relative liver weight and lipid levels were associated with increasing dose levels. Doses of 50 mg/kg or more produced time- and dose-related increases in the incidence of hepatocellular adenomas and carcinomas bearing no causal relationship to the observed renal damage. The first adenoma appeared at week 46; the first carcinoma at week 58. The NTP intends to conduct an audit of the raw data of this study before the final technical report is prepared. The findings of the audit will determine the validity of the

study. The study remains under audit as of June 1985.

9.1.2 Dow Chemical Company Inhalation Study (Rampy et al., 1978)

Two groups of weanling Sprague-Dawley (Spartan substrain) rats, each composed of 96 males and 96 females, were exposed to 600 ppm (4068 mg/m³ air) or 300 ppm (2034 mg/m³ air) of PCE 6 hours/day, 5 days/week, for 52 weeks. An untreated group of 192 males and 192 females served as controls. Controls were not put in inhalation chambers, but were in the treatment room during exposure. At the end of the treatment period of 52 weeks, animals were allowed to survive until sacrifice at 31 months. The composition of the test material (Lot A12282D) by gas chromatographic analysis was as follows: trichloroethylene, 3 ppm (liquid volume %); hexachloroethane, < 12 ppm; carbon tetrachloride, 2 ppm; 4-methyl morpholine, 44 ppm; nonvolatile residue, 2 ppm; PCE, balance. Exposure was done in 3.7 m³ inhalation chambers with a dynamic airflow system. Analyses of PCE levels in the inhalation chambers during the treatment period revealed analytical concentrations (mean $\pm$ standard deviation) of 310 $\pm$ 32 ppm (244 analyses) and 592 $\pm$ 62 ppm (1,245 analyses). Analytical concentrations by infrared analysis within $\pm$ 10% of nominal levels were achieved on 89.3% (low-dose) and 97.1% (high-dose) of the exposure days. Animals were evaluated for clinical toxic signs, body weight changes, urinalysis at 24 months, and hematology at 12 and 24 months. Survivors and decedents were given gross and histopathologic examinations. Bone marrow samples were taken from three males and three females in each group sacrificed at 1 year for cytogenetic evaluation.

Clinical signs of toxicity were not observed with the nominal concentrations of PCE used in this study. Mean body weight gains were similar among groups. Hematology and urine analyses showed no treatment-related effects

of PCE. The mortality patterns exhibited in the study are described in Table 9-2. Mortality in high-dose males was slightly greater than in controls during months 5 to 24; the earlier onset of chronic renal disease in this treatment group was considered to be a contributing factor in increased mortality.

TABLE 9-2. CUMULATIVE SURVIVAL OF SPRAGUE-DAWLEY RATS EXPOSED TO PCE FOR 12 MONTHS

Month of study	0 ppm		300 ppm		600 ppm	
	Males	Females	Males	Females	Males	Females
Initial	189	189	94	91	94	94
6	187	188	94	91	88 ^a	94
12	183	185	91	91	84 ^a	91
18	155	151	74	77	55 ^a	86 ^a
24	44	70	26	37	13 ^a	49 ^a
31	1	12	1	6	1	5

^ap < 0.005 by the Fisher Exact Test.

SOURCE: Adapted from Rampy et al., 1978.

No carcinogenic effects of PCE were observed from pathologic examination of the animals. Statistical analysis of the data showed numerous nonneoplastic abnormalities that occurred spontaneously and were within the normal variation encountered in lifetime studies with this strain of rat. With respect to tumor findings, analysis of the data did not reveal a definite increased tumor incidence in animals exposed to PCE. Tumors or tumor-like changes in the kidney were found in 1/189 control, 2/94 low-dose, and 4/94 high-dose males during

gross necropsy; however, microscopic examination of kidney lesions did not show a statistically significant tumor incidence compared to controls. Although many tumor types were found in treated and control animals, there was no statistically significant ($p > 0.05$) increase in tumor incidence.

The results of this study do not indicate a definite carcinogenic effect of PCE in Sprague-Dawley rats; i.e., the tumor incidence between control and treated rats was similar. However, this study has the following drawbacks: 1) the period of exposure was only 12 months rather than the lifetime of the animals, which would have been a more appropriate duration for carcinogenicity studies; and 2) the dose levels in this study do not appear to have been high enough to provide maximum sensitivity.

9.1.3 Intraperitoneal Administration Study (Theiss et al., 1977)

Theiss et al. (1977) tested PCE for carcinogenicity in the strain A mouse pulmonary tumor induction system. The test sample, a product of the Aldrich Chemical Company, was reagent grade with a purity exceeding 95% to 99%. Strain A/St male mice, 6 to 8 weeks old, were used in this assay. The maximum tolerated dosage, defined as the dosage which five mice tolerated after six intraperitoneal injections over a 2-week period followed by a 4-week observation period, was determined and used in the bioassay. In the main test, 20 mice per treatment group received three intraperitoneal injections of 80, 200, or 400 mg/kg of PCE weekly until total dosages of 1,120, 4,800, and 9,600 mg/kg, respectively, were achieved. Survivors were sacrificed at 24 weeks after the first injection, and the number of surface adenomas was counted. Results were compared with findings in vehicle (tricaprylin) and untreated controls by the Student t test. PCE did not statistically increase ($p > 0.05$) the incidence of pulmonary tumors in this study (Table 9-3). This strain was sensitive to the

positive control chemical urethane, as shown in Table 9-3.

A negative result in this assay is not considered conclusive, since several chemicals known to be carcinogenic in chronic rodent bioassays induce no response in this assay.

TABLE 9-3. PULMONARY TUMOR RESPONSE TO PCE

Compound	Dosage (mg/kg)	No. survivors/ No. animals	No. lung tumors/mouse
Tricaprylin	---	46/50	0.39 ± 0.06^a
PCE	80	15/20	0.27 ± 0.07
	200	17/20	0.41 ± 0.10
	400	18/20	0.50 ± 0.12
Urethan	1,000 mg/kg (1 injection)	20/20	19.6 ± 2.4

^aMean $\pm$ S.E.

SOURCE: Adapted from Theiss et al., 1977.

The strain A mouse pulmonary tumor assay is relatively insensitive to mouse carcinogens for which the effect is confined to the liver (Theiss et al., 1977). For example, chloroform, 2-chloroethyl ether, and hexachlorocyclohexane induce tumors of the liver (not other sites) in mice (NCI, 1975, 1977b; Innes et al., 1969) but were not carcinogenic in the assay by Theiss et al. (1977). The reasons for the negative lung response are not understood, but it may be due to a smaller concentration of activating enzymes in the lung than in the liver.

9.1.4 Skin Painting Study (Van Duuren et al., 1979)

A carcinogenicity study of purified PCE in ICR/Ha Swiss mice was described by Van Duuren et al. (1979). Maximum tolerated dosages were determined in range-finding studies 6 to 8 weeks in duration, and were selected as dosages that did not affect body weight gain or produce clinical signs of toxicity. This study included the following experiments: 1) 30 females were treated topically on the dorsal skin with a single application of 163 mg PCE followed 14 days later by the applications of 5.0 μ g phorbol myristate acetate (PMA) to the skin three times weekly until termination of the study at 428 to 576 days; median survival time was 428 to 576 days. 2) 30 females were given thrice weekly topical applications of 54 mg PCE for the duration of the test (440 to 594 days), with a median survival time of 317 to 589 days. A vehicle (acetone) control group of 30 mice and an untreated control group of 100 mice were included in these experiments.

In the initiation-promotion experiment, 210 mice treated with PMA alone were also on test. The mice were 6 to 8 weeks old at the beginning of the study, and were housed six to a cage. Test sites on the skin were shaved as necessary and were not covered; however, it was the authors' impression that PCE was immediately absorbed and that evaporation from test sites was minimal (personal communication, B. L. Van Duuren, New York University). The animals were weighed monthly, and each animal was examined by necropsy. Tumors and lesions as well as skin, liver, stomach, and kidneys were examined histologically.

PCE did not show initiating activity in the initiation-promotion experiment; the number of mice with skin papillomas (squamous cell carcinomas) was: 4 (0) initiated with PCE, 15 (3) treated with PMA alone, and 0 (0) in the control groups. The study involving repeated application to the skin produced

lung and stomach tumors in 16 and 0 PCE-treated mice, respectively; 11 and 2 vehicle-treated controls, respectively; and 30 and 5 untreated controls, respectively.

The negative results for PCE in mouse skin as an initiator and as a complete carcinogen can be reconciled with the positive mouse liver response in the NCI study if it is hypothesized that the skin does not have the necessary enzymes to convert PCE to an active metabolite, whereas the liver does have this capability.

The lack of sensitivity of skin application tests, as compared with tests using other routes of exposure, is apparent from the results of Van Duuren et al. (1979) with 1-chloroprene, cis-1,3-dichloropropene, and 2-chloropropenal. They found that none of the three compounds induced a response as initiators in initiation-promotion experiments or with repeated topical application on the skin. However, they did observe a statistically significant increase in the incidence of forestomach tumors in female Ha:ICR Swiss mice dosed by gavage with 1-chloroprene ($p < 0.0005$) and 2-chloropropenal ($p < 0.05$) and in the incidence of local sarcomas in female Ha:ICR Swiss mice treated with cis-1,3-dichloropropene ($p < 0.0005$) by subcutaneous injection.

9.2 EPIDEMIOLOGIC STUDIES

There are seven epidemiologic studies either completed or currently in progress that relate to PCE exposure. Only two of these studies, however, have actually identified persons exposed to PCE. Because PCE has been used in the dry-cleaning industry, however, the present discussion also includes three proportionate mortality studies of decedents who had worked in the dry-cleaning industry, as well as two case-control studies in which the cases and controls were asked about their employment histories, including employment in the dry-

cleaning industry.

9.2.1 Kaplan (1980)

Kaplan (1980) did a retrospective cohort mortality study of dry-cleaning workers exposed to PCE for at least one year prior to 1960. The study was performed under contract to the Biometry Section of the National Institute for Occupational Safety and Health (NIOSH) Industry-Wide Studies Branch.

In a preface to the discussion of the study, Kaplan reported that levels of PCE exposure were "much higher" for cleaners (machine operators) than for other employees of dry-cleaning establishments. A geometric mean time-weighted average for PCE was 22 ppm for the machine operators. For all other jobs, the highest corresponding value was reported to be 3.3 ppm. These data were provided through a NIOSH industrial hygiene survey of dry-cleaning facilities.

The study cohort, selected from records maintained by several labor unions, consisted of 1,597 dry-cleaners exposed for more than 1 year prior to 1960. The primary solvent used was PCE. Efforts were made by the author to exclude all persons with previous occupational exposure to carbon tetrachloride or trichloroethylene. By September 30, 1977, the end of the study period, 1,058 individuals were found to be alive, 285 were deceased, and 254 were of unknown vital status. The extent of follow-up varied by sex; 8% of the males and 20.4% of the females remained lost to follow-up. Race was known only for deceased workers, and was obtained from death certificate data. Because of the lack of information regarding race, observed deaths by cause were compared to expected deaths by means of a standardized mortality ratio (SMR) for whites, an SMR for blacks, and a composite point estimate SMR for both. Based on the assumption that every member of the cohort was white, expected deaths for whites were derived by multiplying the person-years accumulated for the entire

cohort by the white death rates within 5-year age groups (separately for males and females and then with the two combined). Expected deaths for blacks were similarly generated by assuming that all cohort members were black. Composite expected deaths were calculated by weighting the total accumulated person-years for the cohort in each 5-year age group by the proportion of person-years attributable to the deceased blacks and the proportion attributable to deceased whites, and then multiplying each total separately by the corresponding death rates for whites and blacks, and finally adding across age groups to get the "composite" expected deaths.

Because death certificates could not be located for all of the deceased, it was assumed that those deaths for which no death certificates could be found had the same distribution by cause as those for which death certificates were available. Thus the SMRs for each cause of death were corrected to reflect the missing death certificates. Using the SMR for deaths from malignant neoplasms of the colon in whites as an example, this correction was made in the following manner:

$$\frac{(11/247 \times 38) + 11}{11} \times \frac{11}{6.98} \times 100 = 182$$

where: 247 is the number of deaths in the cohort with death certificates;
 38 is the number of deaths in the cohort without death certificates;
 11 is the observed number of colon cancer deaths identified by death certificates;
 6.98 is the expected number of white colon cancer deaths;
 100 is a constant used in calculating SMRs (by convention, SMRs are expressed as a factor of 100); and
 182 is the corrected white colon cancer SMR.

No tests of significance of any of the SMRs were done by the author. However, the

author called attention to the elevated SMR from malignant neoplasms of the colon as possibly related to occupational exposure (6.98 white expected deaths, 6.77 black expected deaths, and composite SMR = 182). Using an observed number of colon cancer cases corrected for the loss of death certificates, the Carcinogen Assessment Group (CAG) found that the SMR for either whites or blacks would be statistically significant ($p < 0.05$).

The author points out that because the expected numbers of deaths were calculated using U.S. rates, which include a higher socioeconomic class than the dry-cleaners in this study, and because higher socioeconomic class is associated with a risk of colon cancer, the risk of colon cancer from exposure to PCE found in this study is probably underestimated. Furthermore, the expected number of colon cancer cases in this study was calculated using mortality rates for neoplasms of the intestine, except rectum. Although most of the deaths expected using mortality rates for neoplasms of the intestine would be deaths from malignant neoplasms of the colon, some deaths would be from neoplasms of the small intestine. Since all of the observed deaths were from malignant neoplasms of the colon, the comparison of the observed to expected colon cancer deaths in this study would also tend to underestimate the colon cancer risk from exposure to PCE.

It should also be noted that although it could be argued that the number of observed colon cancer deaths in each of the four union locals in this study was small, an elevated colon cancer SMR did exist in each of the four locals. Finally, it should be noted that the colon cancer SMR appeared to demonstrate a positive correlation with the length of the follow-up period. This last finding must be viewed with a great deal of caution, however, because of the author's difficulty in defining length of exposure and hence length of follow-up.

In addition to colon cancer, the SMRs for cancer of various other sites were also elevated. These included rectum, pancreas, respiratory system, urinary organs, and "other and unspecified sites (major)." None of these were significant at the $p < 0.05$ level when tested using an observed number that was corrected for lost death certificates; however, the SMRs for cancer of three of these sites [respiratory system, urinary organs, and "other and unspecified sites (major)"] could be considered borderline significant ($0.10 < p < 0.05$).

Perhaps the major weakness of this study with regard to evaluating PCE as a carcinogen is that the history of solvent exposure prior to 1960 was unknown for nearly half of the union member shops. Because the majority of dry-cleaning establishments in the United States used petroleum distillates as the primary cleaning agent prior to 1960, it is quite possible that most of the shops in this study used petroleum distillates as the cleaning solvent prior to changing to PCE.

Other important confounding variables were also not controlled. For example, smoking is a major confounding variable to be considered when evaluating a potential risk for respiratory or bladder cancer, both of which were found in excess in this study. Socioeconomic status, as has been discussed, is a confounding variable for colon cancer.

Another weakness of this study is that 16% of the study cohort was lost to follow-up. Currently, NIOSH is attempting to improve the percentage of follow-up as well as to add to the length of follow-up. In addition, NIOSH has identified other individuals who were exposed to PCE for at least one year prior to 1960, so that the size of the cohort has also been increased. The results should be available by 1986.

In summary, this study appears suggestive that dry-cleaning workers exposed to PCE are at an elevated risk of colon cancer mortality. Potential

exposure to petroleum distillates for approximately half of the cohort, however, limits any conclusions with regard to the carcinogenicity of PCE in humans.

9.2.2 Blair et al. (1979)

Blair et al. (1979) reported, as the preliminary results of a cohort study of 10,000 laundry and dry-cleaning workers, a proportionate mortality analysis of 330 of the workers who had died during the period 1957-1977. Deaths were identified from the mortality records of two union locals in St. Louis, Missouri. The distribution by cause of death among the 330 was compared to that expected based on the proportionate mortality experience of the United States. Only 279 of the 330 had worked exclusively in dry-cleaning establishments; however, the authors did not report the proportionate mortality analyses of these 279. Furthermore, the dry-cleaning agent used by the dry-cleaners in this study is unknown. Among the 330 deaths, deaths from cancer of the lung, cervix, and skin contributed to the finding of a significant ($p < 0.05$) excess proportion of deaths from cancer at all sites. For lung cancer, there were 17 deaths observed versus 10 expected ($p < 0.05$); for cervical cancer, 10 observed deaths versus 4.8 expected ($p < 0.05$); and for skin cancer, 3 observed deaths versus 0.7 expected ($p < 0.05$). On the other hand, a significant deficit of deaths occurred in the cause category identified as "all circulating diseases," with 100 observed versus 125.9 expected ($p < 0.005$)--a finding that could account for the excess proportion of cancers observed.

Only limited conclusions can be drawn from this study as to the potential carcinogenicity of PCE. This is true for several reasons. First, the study did not report the distribution of deaths for dry-cleaners alone. Second, the dry-cleaning agent these workers used is not known. Lastly, certain possibly

confounding variables were not considered in the analysis of the results. These variables include smoking (with regard to the lung cancer excess), socioeconomic status (with regard to the cervical cancer excess), and sunlight exposure (with regard to the skin cancer excess).

9.2.3 Katz and Jowett (1981)

Katz and Jowett (1981) analyzed the death certificate records of 671 white female laundry and dry-cleaning workers who had died during the period 1963-1977. The records of dry-cleaning workers were not studied separately from those of laundry workers, since both groups of workers shared the same occupational code. Furthermore, it is not known whether those decedents who were dry-cleaners used PCE as a dry-cleaning agent. During the 1940s, petroleum derivatives were the predominant dry-cleaning agents used in the United States. A shift to the use of PCE began in the late 1940s and gained momentum in the 1950s and 1960s. By 1977, approximately 75% of the dry-cleaning plants in the United States were using PCE. However, in the period before 1960, petroleum distillates were still the dominant solvents in use (Kaplan, 1980).

In this study, cause-specific proportionate mortality for the 671 deceased laundry and dry-cleaning workers was compared to that for the deaths of all other working females in Wisconsin during the same period, and to deaths of females in "lower-wage occupations" in Wisconsin during the same period. Significantly elevated proportionate mortality ratios (PMRs) were found for deaths from cancer of the genitals (unspecified) ($p < 0.01$) and for deaths from cancer of the kidney ($p < 0.05$) when deaths among women of all occupations and deaths among women of "lower-wage occupations" were used as the comparison groups. In summary, this study, although suggesting an association of employment in the dry-cleaning industry with excess risks of certain types of cancer,

cannot be said to demonstrate that PCE exposure presents a carcinogenic risk because of the possible confounding influence of other dry-cleaning agents, and because of the fact that no distinction was made between laundry and dry-cleaning workers.

9.2.4 Lin and Kessler (1981)

Lin and Kessler (1981) did a case-control study of 109 pancreatic cancer cases diagnosed during the period 1972-1975 from 115 hospitals in five metropolitan areas. The control group was composed of subjects who were free from cancer but who were similar to the study group in age (± 3 years), sex, race, and marital status, and who had been selected at random from among contemporaneous admissions to the same hospital. Cases and controls were asked about demographic characteristics, residential history, toxic exposures, animal contacts, smoking habits, diet, medical history, medications, and family history. Males were asked about sexual practices and urogenital conditions. Females were questioned on these topics and also on their marital, obstetric, and gynecologic histories. Among other statistically significant associations, an association was found between pancreatic cancer and employment either as a dry-cleaner or in a job involving exposure to gasoline. It is not known, however, how many individuals with pancreatic cancer were employed as dry-cleaners and how many were employed in occupations involving exposure to gasoline. Furthermore, the dry-cleaners may have used a variety of dry-cleaning agents other than PCE. Thus this study, although suggestive of an association between employment as a dry-cleaner and an excess risk of pancreatic cancer, cannot be said to demonstrate an association between pancreatic cancer and PCE exposure.

9.2.5 Duh and Asal (1984)

Duh and Asal (1984) analyzed the death certificates of 440 laundry and

dry-cleaning workers who had died in Oklahoma during the period 1975-1981. Occupations were identified from the death certificates. Laundry workers could not be separated from dry-cleaning workers because they shared similar occupational codes. Age, sex, race, and cause distribution of deaths in the United States in 1978 were used as the standards of comparison. An analysis was done using the Standardized Mortality Odds Ratio (SMOR) (Miettinen and Wang, 1981). The SMOR differs from the more traditional Proportionate Mortality Ratio (PMR) in that it compares the number of deaths from the cause of interest to the number of deaths from auxiliary causes in the exposed population (the odds) to the expected odds derived from a comparison population, taking into account covariates such as age, sex, and race. The authors used "other diseases" and "all circulatory diseases" as auxiliary causes for the analyses, and indicated that the results were almost the same as that of the PMR method when "all other diseases" were used as auxiliary causes, but they differed on two points when "all circulatory diseases" were used as auxiliary causes. (The authors did not indicate what these two points were.) The SMOR for homicide was the only SMOR in the study group that was significantly elevated for non-cancer causes of death (SMOR = 3.8, 95% confidence interval (CI) = 1.4-10.6; observed deaths = 7, expected deaths = 2.9). Deaths from ischemic heart disease were significantly decreased (SMOR = 0.8, 95% CI = 0.7-1.0; observed = 134, expected = 153.7). Among deaths from cancer, the SMORs for cancer of the respiratory system (SMOR = 1.8, 95% CI = 1.3-2.5; observed = 39, expected = 23.8); cancer of the lung (SMOR = 1.7, CI = 1.2-2.5; observed = 37, expected = 22.6); and cancer of the kidney (SMOR = 3.8, 95% CI = 1.9-7.6; observed = 7, expected = 1.9) were significantly elevated. Deaths from cancer of the breast were significantly decreased (SMOR = 0.1, 95% CI = 0.0-0.4; observed = 1, expected = 10.5). Since, as the authors noted, wage levels in the laundry and dry-

cleaning industry are not high, the excess of homicide and the deficit of breast cancer and ischemic heart disease may have resulted from the inverse correlation with socioeconomic status.

Smoking histories were not available for any of the death certificates analyzed. Because both lung and kidney cancer have been associated with smoking, it is difficult to evaluate the study's elevated SMORs for those diseases with respect to dry-cleaning exposures. The authors did note, however, that mortality from other smoking-related diseases such as emphysema, ischemic heart disease, cancer of the buccal cavity, and pharyngeal cancer was slightly less than expected.

As the authors noted, there may have been a bias in the analysis because the occupational code that identified the study group included both laundry and dry-cleaning workers. Such a bias would tend to weaken any association that might have been present by adding to the group at potential risk a number of individuals that would not otherwise have been considered exposed.

Of interest is the authors' report that petroleum solvents accounted for greater than 50% of the dry-cleaning solvents used in Oklahoma in 1983. As with several of the other epidemiologic studies reviewed here, this study suggests that dry-cleaners and/or laundry workers may be at an excess risk of cancer. However, the possible confounding effects of both petroleum solvents and smoking, and the fact that dry-cleaners could not be separated from laundry workers in the analysis, preclude the conclusion that PCE exposure is associated with an excess risk of cancer.

9.2.6 Asal (personal communication, 1985)

Dr. Nabih Asal of the University of Oklahoma School of Public Health is currently analyzing the data from a kidney cancer case-control study using both

hospital and population controls. A variety of potential risk factors are being evaluated including occupation. While information will not be available from this study on cases and controls who worked in the dry-cleaning industry with regard to exposure to specific dry-cleaning solvents, Dr. Asal is also conducting a cohort study of dry-cleaning establishment owner-operators. Data on solvent use is extensive for the different dry-cleaning establishments, and thus it is hoped that this study will provide useful data with which to evaluate PCE with regard to carcinogenicity.

9.3 RISK ESTIMATES FROM ANIMAL DATA

The evidence for the carcinogenicity of PCE serving as the basis for the quantitation of risks consists of a single positive mouse study (NCI, 1977a). In this study, there was a statistically significant increase in the incidence of malignant tumors of the liver in both sexes of B6C3F1 mice.

Because of a question regarding the possible presence of contaminant epoxides in PCE ($< 0.05\%$ the maximum possible), the assay was repeated (NTP, 1983, draft) with PCE without detectable amounts of epoxides, although at lower dose levels. Doses of 50 mg/kg to 200 mg/kg produced time- and dose-related increased incidences of hepatocarcinomas. At the present time, the NTP final report is not available.

The cancer data from epidemiologic studies are inconclusive. The evidence for mutagenicity of PCE is also inconclusive. However, the more conservative public health view would regard PCE as a possible human carcinogen. The calculations in this document for estimating, in quantitative terms, the impact of PCE as a carcinogen are made independently of the overall weight of evidence for the carcinogenicity of PCE. The calculations are made as if PCE were a human carcinogen.

9.3.1 Selection of Animal Data

For some chemicals, several studies in different animal species, strains, and sexes, each run at several doses and different routes of exposure, are available. A choice must be made as to which of these data sets to use in the mathematical extrapolation model. It may also be appropriate to correct for metabolic differences between species and for different routes of administration.

For PCE, only the data from the NCI (1977a) oral gavage lifetime study in male and female mice, showing a significant excess of tumors (hepatocellular carcinomas) have been used. Table 9-4 gives the time-weighted average dosage and tumor incidence (at "low" and "high" doses) in male and female B6C3F1 mice for the NCI study. PCE in corn oil was given, once a day, by gastric intubation, 5 days/week, starting at 25 days of mouse age and continuing for 78 weeks. During the final 26 weeks, doses were given in a cyclic pattern of one week without treatment followed by four weeks with treatment. After the 78-week treatment period, the mice were given an additional 12 weeks before sacrifice.

Taking into account differing dosage levels, male and female mice appear to be equally susceptible to a carcinogenic response to PCE. Furthermore, since the "low" dose and "high" dose response did not differ statistically, a plateauing of the response is indicated. Hepatic tumors (0-10%) were not detected in untreated or vehicle-treated groups until 90-91 weeks. For treated male mice, tumors were detected at 27 weeks in the "low" dose group and 40 weeks in the "high" dose group, and for treated female mice at 41 weeks in the "low" dose group and 50 weeks in the "high" dose group.

TABLE 9-4. INCIDENCE OF HEPATOCELLULAR CARCINOMAS IN MALE AND FEMALE B6C3F1 MICE FED PCE BY GAVAGE

Sex and no. mice	Median survival (weeks)	Average terminal weight (g)	Time-weighted average gavage dose ^a		Incidence
			mg/kg/day	mg/animal/day	
Males					
20	>90		untreated	0	2/17 (12%)
20	>90	30	0; vehicle ^b	0	2/20 (10%)
50	78		536	16.1	32/49 (65%)
50	43		1,072	32.2	27/48 (56%)
Females					
20	>90		untreated	0	2/20 (10%)
20	>90	25	0; vehicle	0	0/20 (0%)
50	62		386	9.7	19/48 (40%)
50	50		1,172	19.3	19/48 (40%)

^aSum (dose in mg/kg x no. of days at that dose)

^bSum (no. of days receiving any dose)
Corn oil.

SOURCE: NCI, 1977a.

9.3.2 Interspecies Dose Conversion

9.3.2.1 General Considerations--Scaling of toxicologic effects, including carcinogenicity, among species has been elaborated in several published papers (Freireich et al., 1966; Gillette, 1976; Krasovski, 1976; Dedrick, 1973; Rall, 1977; Dedrick and Bischoff, 1980; and Gehring et al., 1980). The major components requiring consideration in determining an appropriate extrapolation base for scaling carcinogenicity data from laboratory animals to man are: 1) toxicologic data, 2) metabolism and kinetics, and 3) covalent binding. The published literature concerning the biological basis for extrapolation of the dose-carcinogenic response relationship of laboratory animals to man has been reviewed in a paper prepared by Davidson (1984) for the Carcinogen Assessment Group and presented by Parker and Davidson (1984).

9.3.2.1.1 Toxicologic Data. Across species (mice, rabbit, cat, dog, and man) the acute LD₅₀ of PCE is similar (Tables 6-1 and 6-2; Chapter 6). This similarity is most probably due to an equally effective concentration, leading to central nervous system depression and resulting in respiratory depression and death. While other toxicologic end points (modalities of liver and kidney damage) have been measured (Klassen and Plaa, 1967), these measures are difficult to evaluate in the numerous and separate studies, and comprehensive comparative studies have not been conducted across species using the same toxicologic end point.

9.3.2.1.2 Metabolism and Kinetics. It is generally accepted that the liver and kidney toxicity, and carcinogenicity potential of the halogenated ethylenes relate to the metabolic conversion of these compounds to biologically reactive intermediates (reviewed by Bolt et al., 1982). All of the chlorinated ethylenes are initially biotransformed by microsomal monooxygenases to epoxide intermediates which bind to cellular macromolecules, are molecularly rearranged

to less reactive species, or are modified by conjugation through the "detoxification mechanisms." Bolt et al. (1982) postulated that the reactivities and hence toxicities of the individual epoxides formed from chlorinated ethenes depend on the type of chlorine substitution. Symmetric substitution such as is seen with PCE renders the epoxide relatively more stable and therefore less reactive, while asymmetric substitution results in highly unstable reactive epoxides (e.g., vinyl chloride). However, tumors may arise in animals by genetic or epigenetic mechanisms in which recurrent cytotoxicity may lead to the development of cancer. For example, the accumulative effect of even minimally reactive metabolites may produce cancer. Therefore, the extent of metabolic activation of these metabolites, across species, provides a comparative index of cellular toxicity and a basis for determining a "mouse to man" extrapolation scaling factor. Moreover, such a comparison provides one index in the evaluation of the "susceptibility" of a species (e.g., mouse versus rat).

The metabolism and kinetics of PCE have been studied in three species: mouse, rat, and man. The principal end metabolites of PCE that have been reported as common to these species are CO_2 and the urinary metabolites trichloroacetic acid (TCA) and oxalic acid. Pegg et al. (1979) specifically identified the major urinary metabolite in rats as oxalic acid. There is strong evidence that PCE oxide is formed by mixed-function oxidase (MFO) systems as a precursor of these metabolites, and the additional reactive intermediate trichloroacetyl chloride as a precursor of TCA (Bolt et al., 1982). In general, all of the end metabolites have been poorly identified and quantified in mouse, rat, and man, and there is no comparative experimental evidence that the metabolic pathway(s) for PCE qualitatively differs for these species.

9.3.2.1.2.1 Oral Studies. Comparative balance studies for the disposition of PCE (extent of metabolism) have been carried out after both oral and inhalation exposure in both rats and mice. The oral exposure studies are the most pertinent to the conditions of the NCI-PCE bioassay.

Pegg et al. (1979) and Schumann et al. (1980) administered ^{14}C -PCE in corn oil vehicle as single intragastric doses of 1 and 500 mg/kg to Sprague-Dawley rats and B6C3F1 mice. ^{14}C -radioactivity was determined for exhaled breath, urine, feces, and carcass for 72 hours following dosing. In addition, pulmonary excretion of unchanged PCE was measured. The data from these two studies are collated and re-expressed in milligram equivalent units in Table 9-5, thus allowing comparison of the metabolism of PCE in the rat and mouse.

As indicated in Table 9-5, the recovery (95-97%) of ^{14}C -radioactivity in these experiments indicates that virtually complete absorption of PCE from the gastrointestinal tract occurs in the rat and mouse. In the rat, peak blood concentration of PCE occurred 1 hour after oral dosing; disappearance followed apparent first-order kinetics with a half-life of 6 hours (a reflection of first-order kinetics for pulmonary excretion and not for metabolism). This relatively long half-life suggests that PCE doses are not eliminated completely from the body in less than 24 to 30 hours.

For rats, oral doses of 1 and 500 mg/kg were metabolized 29% (0.07 mg) and 10% (12.52 mg) respectively, while 71% and 90% of the doses were excreted unchanged through the lungs. Thus, even at low doses (1 mg/kg) the metabolism of PCE is limited and saturable. For mice, the metabolism of PCE can be compared to rats at the 500 mg/kg dose. Mice metabolized 17% (1.75 mg) of this dose and rats 10% (12.52 mg). In the rats, the greater portion of the dose (83%) was eliminated unchanged through the lungs, indicating limited metabolism in this species also. These observations of very limited metabolism by rats

TABLE 9-5. DISPOSITION OF ^{14}C -PCE RADIOACTIVITY FOR 72 HOURS AFTER SINGLE ORAL DOSES TO SPRAGUE-DAWLEY RATS AND B6C3F1 MICE

	Rats (av. of 3) ^a		Mice (av. of 3)
	1 mg/kg (0.25 mg/animal) Mg-eq per animal	500 mg/kg (125 mg/animal) Mg-eq per animal	500 mg/kg (12.25 mg/animal) Mg-eq per animal
Expired unchanged	0.174 (71%)	110.67 (90%)	8.90 (83%)
Metabolized			
$^{14}\text{CO}_2$	0.007	0.57	0.14
Urine	0.040	5.72	1.53
Feces	0.015	4.82	0.13
Carcass	0.008	1.41	0.05
	0.070 (29%)	12.52 (10%)	1.85 (17%)
Total	0.244	123.19	10.75

^aBased on average experimental animal weight (grams): 250, rat; 24.5, mouse.

SOURCES: Adapted from Pegg et al., 1979 and Schumann et al., 1980.

and mice are in accord with those of Filser and Bolt (1979) who, for the rat, estimated V_{max} at less than 1.2 mg/kg/hour. Using this estimate for a 250-g rat, 7 mg of an absolute dose of 125 mg (500 mg/kg) would be expected to be metabolized in 24 hours. This compares favorably with the 12.52 mg experimental value given in Table 9-5.

The disposition of the oral dose of 500 mg/kg to rats and mice is comparable to the oral doses given to mice in the NCI bioassay of PCE (386 to 1,072 mg/kg; Table 9-4). The ratio of the dose metabolized per animal for the rat versus mouse from Table 9-5 is as follows:

<u>Dose</u>	<u>Amount metabolized ratio: rat/mouse</u>
500 mg/kg	12.52/1.85 = 6.77

The body weight ratios for the allometric extrapolation bases $W^{0.67}$ (surface area) and $W^{1.0}$ (mg/kg) are:

$$W^{0.67} = (250/24.5)^{0.67} = 4.74$$

$$W^{1.0} = (250/24.5) = 10.20$$

Hence, the comparative metabolism of PCE for the rat and mouse is more consistent with metabolism proportional to surface area ($W^{0.67}$) than to body weight.

The long half-life of PCE (6-7 hours) in the rat or man (55-65 hours) suggests that for daily dosing (24-hour interval) metabolism continues throughout the entire interval between successive exposures. Therefore, 1) the daily exposure to PCE represents chronic administration rather than repetitive dosing in which the dose is completely cleared from the body before the next dose, as is the case for other chloroethylenes with short half-lives (e.g., trichloro-

ethylene, vinylidene chloride); and 2) the "repair" mechanisms are constantly overshadowed by metabolism.

Buben and O'Flaherty (1985) examined the extent of PCE metabolism in male mice during subchronic administration by gavage in corn oil vehicle for 6 weeks (5 days/week; conditions similar to the NCI bioassay of PCE). While the strain of mice was Swiss-Cox and not the B6C3F1 used in the NCI bioassay, the results in this study are probably relevant and generally applicable. The investigators found that PCE metabolism in mice was capacity-limited and dose-dependent. Metabolism was measured by urinary metabolite(s) excreted per day. TCA was found to be the only urinary metabolite. Oxalic acid was not considered to be a significant PCE metabolite, since the oxalic acid excreted in treated and control animals was comparable. However, labeled PCE was not used, and hence there was no assurance that a portion of the oxalic acid excreted was not derived from PCE, as was found for the rat by Schumann et al. (1980) and for mice by Yllner (1961). Because urinary TCA was the sole measure of metabolism in the Buben and O'Flaherty (1985) study, it is judged that this study represents somewhat of an underestimate of total metabolism, since other metabolites, including CO₂ in exhaled air, tissue-bound metabolites (Schumann et al., 1980; Pegg et al., 1979), and metabolite(s) in feces (< 5%; oxalic acid, etc.), were not taken into account. Nonetheless, urinary TCA can be expected to represent 70% to 80% of the amount of PCE metabolized. Daily urinary TCA tended to increase in amount during each week of treatment, with leveling (plateauing) towards the end of the week, presumably because of the relatively long half-life of PCE. Urine collections (24-hour) were taken at the plateau or steady-state metabolism.

Figure 9-5 shows the dose-dependent relationship between administered dose (mg/kg/day) and excreted urinary metabolite (mg TCA/kg/day). This hyperbolic

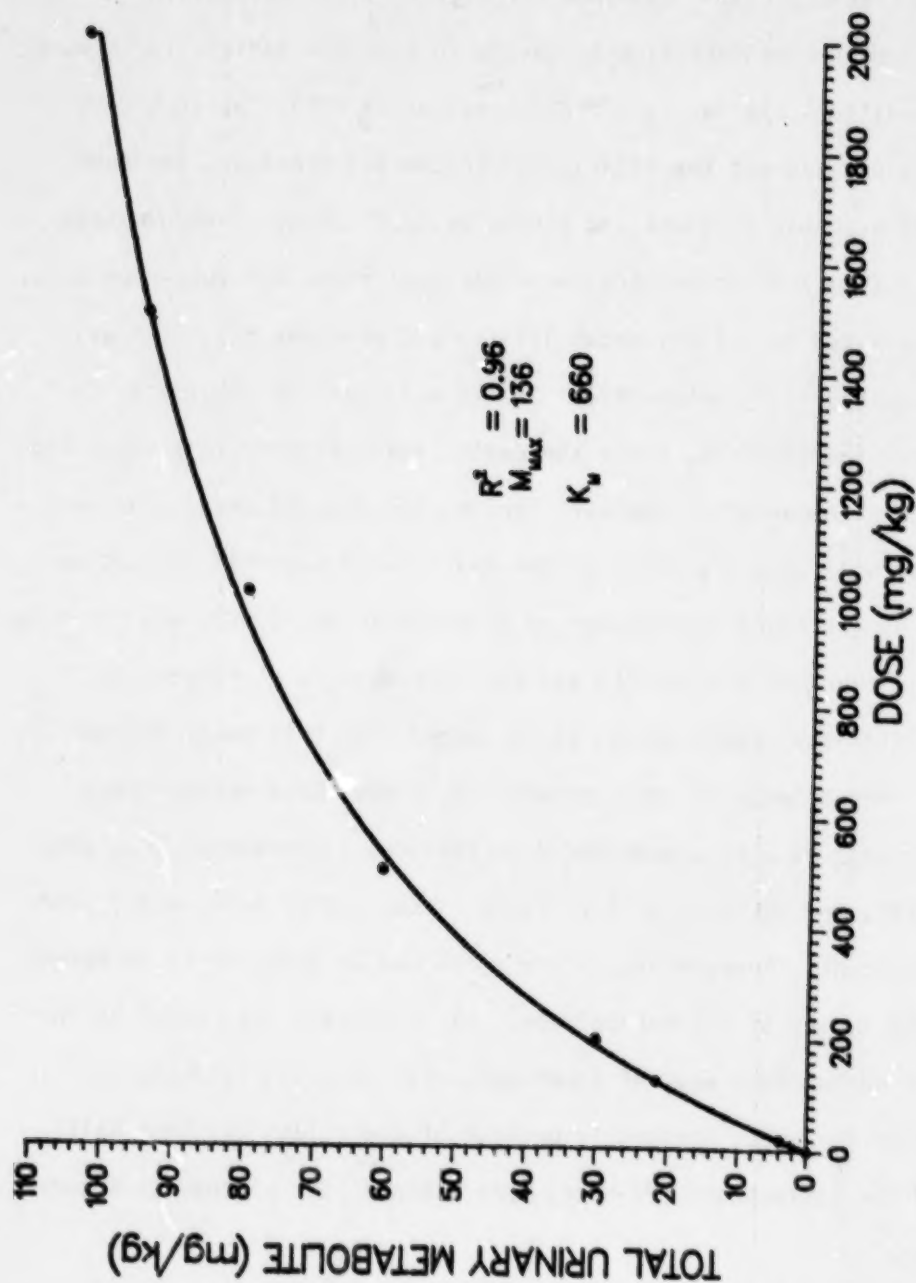


Figure 9-5. Relationship between the PCE dose and the amount of total urinary metabolite excreted per day by mice in each group.

SOURCE: Buben and O'Flaherty, 1985.

curve fitted well to the Michaelis-Menten equation with a $V_{\max}$ of 136 mg PCE/kg/day and a K_m of 660 mg/kg. The curve deviates from linearity above 200 mg/kg doses, indicating that even at "low" doses the metabolism of PCE is capacity-limited in mice. Schumann et al. (1980) and Pegg et al. (1979, Table 2) found that for a single 500 mg/kg dose to B6C3F1 mice, 75.5 mg PCE/kg was metabolized. From Figure 9-5, for a comparable dose 60 mg TCA/kg was observed. On a molar equivalent basis, these two metabolized doses agree surprisingly well (455 μ moles PCE versus 367 μ moles TCA, where 1 μ mole PCE is metabolized to 1 μ mole TCA). This indicates that about 80% of the metabolism is represented by urinary TCA, i.e., measuring only the TCA excretion of the mice probably underestimates total metabolism by about 20%. (Milligrams of urinary TCA is essentially equivalent to milligrams of PCE metabolized, since the molecular weight of TCA is 163.40 and that of PCE is 165.85.) Finally, an important observation can be made from the data of Buben and O'Flaherty (Figure 9-5) relevant to the NCI bioassay. The NCI gavage averaging doses were for male mice, 536 and 1,072 mg PCE/kg/day, and for female mice, 386 and 772 mg PCE/kg/day. The amount of metabolites contributing to a carcinogenic response can be estimated as follows from Figure 9-5 (assuming that Swiss-Cox mice and B6C3F1 mice metabolize PCE similarly):

	<u>NCI gavage dose</u>	<u>Urinary metabolite from Fig. 9-5</u>	<u>% increase with dose</u>
	mg/kg/day	mg TCA/kg/day	
Males	536	60.95	38
	1,072	84.18	
Females	386	50.19	46
	772	73.32	

Thus, for a 100% increase in PCE administered doses, metabolism increases only 38% to 46%. This observation may account in part for the failure to observe a dose-related tumor increase in mice in the NCI study (Table 9-4). The NTP bioassay used lower doses of 50 to 200 mg/kg, and dose-related increases of hepatocarcinomas in mice were found. This is consistent with the dose-dependent findings given in Figure 9-5, since metabolism contributing to tumorigenic response increases proportionally greater for low gavage doses than for higher doses. In fact, Figure 9-5 shows nearly a direct linear relationship between amount of dose metabolized and dose administered below 200 mg/kg.

Finally, it is important to note that Buben and O'Flaherty (1985) demonstrated that indices of PCE hepatotoxicity (increased liver weight, liver triglyceride accumulation, glucose-6-phosphatase activity, and serum SGPT activity) were highly correlative with amount of PCE metabolized by mice. The degree of liver response, as measured by each toxicity parameter when plotted against total urinary metabolites, was linear in all cases, suggesting that the hepatotoxicity of PCE in mice is directly related to the metabolism of PCE.

9.3.2.1.2.2 Inhalation Studies. Pegg et al. (1979) and Schumann et al. (1980) have also determined body burdens and metabolism of Sprague-Dawley rats and B6C3F1 mice after inhalation exposure to 10 and 600 ppm PCE for 6 hours. The animals were exposed to ^{14}C -PCE and the radioactivity determined in urine, feces, expired air, etc., and unchanged ^{14}C -PCE in expired air for 72 hours postexposure. The data from the Pegg et al. and Schumann et al. studies are collated and re-expressed for comparison of rat and mouse metabolism in Table 9-6.

For rats exposed to 10 or 600 ppm, only 32% (0.47 mg) and 12% (9.11 mg) respectively, of the estimated PCE uptake was metabolized. The remaining PCE

TABLE 9-6. DISPOSITION OF ^{14}C -PCE RADIOACTIVITY FOR 72 HOURS AFTER INHALATION EXPOSURE FOR 6 HOURS
TO SPRAGUE-DAWLEY RATS AND B6C3F1 MICE

	Rats (av. of 3) ^a		Mice (av. of 3)
	10 ppm	600 ppm	10 ppm
Expired unchanged	1.008 (68%)	68.39 (88%)	0.048 (12%)
Metabolized			
$^{14}\text{CO}_2$	0.053	0.54	0.032
Urine	0.275	4.54	0.285
Feces	0.076	2.36	0.027
Carcass	0.063	1.67	0.012
	0.467 (32%)	9.11 (12%)	0.356 (88%)
Total	1.475	77.50	0.404

^aAverage experimental animal weights (grams): 250, rat; 24.5, mouse.

SOURCES: Adapted from Pegg et al., 1979 and Schumann et al., 1980.

uptake, 68% and 88%, was excreted via the lungs unchanged. Thus, limited and saturable metabolism of PCE is evident. For mice, the metabolism of PCE can be compared to rats only at the low exposure of 10 ppm exposure concentration. The percentage of uptake metabolized was considerably more extensive (88% of the dose), and only 12% was excreted unchanged. However, the absolute amount of dose metabolized is comparable at 10 ppm between mice and rats. These results suggest that PCE metabolism is limited and saturable in these species and confirm similar observations after oral exposure.

The data of Table 9-6 from inhalation studies, when compared with that of Table 9-5 from oral studies, indicated that, for the rat, inhalation of PCE at 600 ppm for 6 hours resulted in 9.1 mg of compound metabolized. This amount is comparable to that of an oral 125 mg/kg dose (12.5 mg). Thus, inhalation exposure to 600 ppm for 6 hours is approximately equivalent to an oral dose of 125 mg/kg.

For mice, the metabolism of PCE was determined only at 10 ppm exposure for 6 hours. The amount metabolized, 0.36 mg, is comparable to the amount metabolized in rats, 0.47 mg.

9.3.2.1.2.3 Inhalation Exposure in Humans. PCE is readily absorbed through the lungs into the blood by first-order passive diffusion processes. Two major processes account for the known elimination of PCE from the body: 1) pulmonary excretion of unchanged PCE and 2) metabolism of PCE to urinary metabolites. The metabolism of PCE appears to be very limited in humans, as it is in experimental animals. Only a small percentage of the estimated amounts absorbed by inhalation are metabolized to TCA and other chlorinated metabolites found in the urine (see Chapter 4). Saturation of metabolism has been estimated to occur in humans at relatively low exposure concentrations (between 100 and 400 ppm PCE in inspired air--see Chapter 4). Estimates of the

extent of metabolism in humans have been made from balance studies by measuring urinary metabolites after accounting for a retained inhalation exposure dose (see Chapter 4). However, there are several difficulties associated with balance studies in humans. For example, problems are encountered in obtaining an accurate measurement of the retained dose of PCE from inhalation exposure. Balance studies in humans are obviously incomplete. One shortcoming in some of the older PCE studies is the imprecision of the analytical methodologies; for example, the Fujiwara reaction for measuring metabolites. Also, urinary sampling may not have been sufficient considering the long half-life of PCE metabolites. In addition, individual variations among subjects can be rather high. Only urinary TCA was used in these studies as a measure of metabolism, and the possibility exists that significant amounts of metabolites other than TCA are excreted in the urine or by other routes. It has been proposed that a much higher percentage of the dose is actually metabolized, and that a so-far unrecognized pathway may exist for PCE and for other metabolites which have not yet been identified. However, measuring the amount of urinary TCA is one approach that may be used to compare the metabolized dose of PCE among species. The metabolized dose may be considered to be the effective dose in organ toxicity and carcinogenicity.

9.3.2.1.3 Covalent Binding. Reactive metabolites of PCE have been shown to bind irreversibly to cellular macromolecules in vitro (Costa and Ivanetich, 1980), and in vivo (Pegg et al., 1979; Schumann et al., 1980). Pegg et al. (1979) exposed rats by inhalation to 10 and 600 ppm ^{14}C -PCE for 6 hours and to oral doses of 1 and 500 mg/kg. For rats exposed by inhalation, hepatic irreversible binding of ^{14}C -PCE occurred in a ratio of bound/total metabolized of 0.72 - 0.87; the turnover of bound radioactivity was slow as measured over 72

hours postexposure. There was no significant difference in the ratio between inhalation and oral exposure, or between low and high doses, showing that binding occurs proportionally to the amount metabolized (Tables 9-5 and 9-6).

Schumann et al. (1980) compared the irreversible binding in vivo for rats and mice exposed to ^{14}C -PCE by gavage (500 mg/kg) as in the NCI bioassay. Their results are shown in Table 9-7. Peak binding to liver protein occurred at 6 hours for mice and 6 to 12 hours for rats, with binding in mice four- to eightfold greater than in rats. These results are in accord with PCE metabolism in these species (Tables 9-5 and 9-6). Schumann et al. found no detectable binding of high specific activity ^{14}C -PCE to liver DNA when mice were exposed by inhalation (600 ppm) or gavage (500 mg/kg). However, the limit of detection was calculated to be 10 to 14.5 alkylations per 10^6 nucleotides, and therefore very low levels of DNA binding may have occurred. These investigators also chronically administered PCE (11 daily doses) by gavage at increasing doses to 1,000 mg/kg. Mice, but not rats, showed a 20% to 25% increase of liver/body weight ratio. However, the DNA/g of liver protein decreased, indicating hypertrophy rather than hyperplasia. Nonetheless, DNA synthesis (^{14}C -thymidine incorporation) increased in both mice and rats in a dose-related manner, indicating activation of "repair" mechanisms. These investigators interpreted their data as demonstrating cytotoxicity (mouse more susceptible than rat) enhancing spontaneous incidence of liver tumors, rather than to genotoxicity. However, the present evidence is insufficient to support one mechanism over another.

9.3.2.2 Calculation of Dose Metabolized from Animal Data

9.3.2.2.1 Method 1. In the NCI study, the time-weighted average gavage dose in corn oil was 536 and 1,072 mg/kg/day for male mice, and 386 and 772 mg/kg/day for female mice; or, based on the average terminal weights, 16.1 and 32.2

TABLE 9-7. TOTAL HEPATIC MACROMOLECULAR BINDING FOLLOWING ORAL EXPOSURE OF RATS AND MICE TO 500 MG/KG ^{14}C -PCE

Time (hours)	$\mu\text{mole of PCE-bound/g hepatic protein}$		
	Mean $\pm$ S.D.		A/B
	A - Mice	B - Rats	
1	0.9643 $\pm$ 0.1154	0.1699 $\pm$ 0.0251	5.7
6	2.7168 $\pm$ 0.5333	0.3310 $\pm$ 0.0271	8.2
12	1.6868 $\pm$ 0.1119	0.4017 $\pm$ 0.0860	4.2
24	1.3862 $\pm$ 0.2622	0.6120 $\pm$ 0.0269	2.3
48	1.0812 $\pm$ 0.2895	0.3474 $\pm$ 0.0595	3.1
72	0.4723 $\pm$ 0.1310	0.3210 $\pm$ 0.0601	1.5

SOURCE: Schumann et al., 1980.

mg/male mouse/day, and 9.7 and 19.3 mg/female mouse/day (Table 9-4). For a comparable dose of 500 mg/kg (12.25 mg/animal) in corn oil, virtually complete absorption was observed (Schumann et al., 1980; Table 9-5). Therefore, it can be assumed that the PCE doses given by gavage in the NCI study were completely absorbed. However, a dose-dependent portion of the oral dose is excreted via the lungs unchanged (83% for a 500 mg/kg dose) and only a fraction is metabolized (17%). Since the percent of the dose exhaled unchanged increases with increasing dose (Table 9-5), and percent metabolized decreases, these values represent a conservative estimate (overestimate) for any higher dose of the NCI bioassay. Hence, the daily body burdens (amount of dose metabolized contributing to carcinogenic response) for the assay mice are given in Table 9-8.

9.3.2.2.2 Method 2. The estimations of PCE body burden metabolized and contributing to a tumorigenic response may be further refined using the data of Buben and O'Flaherty (Figure 9-5) describing the relationship between gavage dose and amount metabolized. Two reservations should be noted: 1) an assumption is made that the relationship determined with Swiss-Cox mice is also appropriate for B6C3F1 mice of the NCI bioassay, and 2) the relationship underestimates metabolism (by about 20%) since only urinary TCA excretion was used as an index of total metabolism (see discussion in Section 9.3.2.1.2.1). However, at the doses of the NCI bioassay (male mice, 536 and 1,072 mg/kg and female mice, 386 and 772 mg/kg) urinary excretion is by far the major route of metabolized compound, and therefore total urinary metabolites should be a reasonable approximation of the amount of metabolism. Furthermore, the experimental conditions of chronic daily administration of PCE by gavage closely mimic the conditions of the NCI bioassay. The daily body metabolic load (amount of dose metabolized contributed to carcinogenic response, as calculated from the relationships of Figure 9-5) for the NCI bioassay are given in Table 9-9.

TABLE 9-8. LIFETIME AVERAGE DAILY EXPOSURE (LAE) FOR B6C3F1 MICE IN THE NCI BIOASSAY, ASSUMING 17% OF DOSE IS METABOLIZED

	Gavage dose (mg/kg/day)	Dose metabolized (mg/kg/day)	LAE ^a (mg/kg/day)
Males	536	91.12	56.41
	1072	182.24	112.82
Females	386	65.62	40.62
	772	131.24	81.24

^aThe LAE of the mice is given by:

$$\frac{78 \text{ weeks}}{90 \text{ weeks}} \times \frac{5 \text{ days}}{7 \text{ days}} \times \text{dose metabolized (mg/kg/day)}$$

SOURCE: NCI, 1977a.

TABLE 9-9. LIFETIME AVERAGE DAILY EXPOSURE (LAE) FOR B6C3F1 MICE IN THE NCI BIOASSAY, USING A DOSE-METABOLISM (IN URINARY EXCRETION) RELATIONSHIP

	Gavage dose (mg/kg/day)	Dose metabolized (mg/kg/day)	LAE ^a (mg/kg/day)
Male mice	536	60.95	37.73
	1072	84.18	52.11
Female mice	386	50.19	31.07
	772	73.32	45.39

^aThe LAE of the mice is given by:

$$\frac{78 \text{ weeks}}{90 \text{ weeks}} \times \frac{5 \text{ days}}{7 \text{ days}} \times \text{dose metabolized (mg/kg/day)}$$

SOURCE: Buben and O'Flaherty, 1985.

9.3.3 Choice of Risk Model

9.3.3.1 General Considerations--The unit risk estimate for PCE represents an extrapolation below the dose range of experimental data. There is currently no solid scientific basis for any mathematical extrapolation model that relates exposure to cancer risk at the extremely low concentrations, including the unit concentration given above, that must be dealt with in evaluating environmental hazards. For practical reasons the correspondingly low levels of risk cannot be measured directly either by animal experiments or by epidemiologic studies. Low-dose extrapolation must, therefore, be based on current understanding of the mechanisms of carcinogenesis. At the present time the dominant view of the carcinogenic process involves the concept that most cancer-causing agents also cause irreversible damage to DNA. This position is based in part on the fact that a very large proportion of agents that cause cancer are also mutagenic. There is reason to expect that the quantal response that is characteristic of mutagenesis is associated with a linear (at low doses) nonthreshold dose-response relationship. Indeed, there is substantial evidence from mutagenicity studies with both ionizing radiation and a wide variety of chemicals that this type of dose-response model is the appropriate one to use. This is particularly true at the lower end of the dose-response curve; at high doses, there can be an upward curvature, probably reflecting the effects of multistage processes on the mutagenic response. The linear nonthreshold dose-response relationship is also consistent with the relatively few epidemiologic studies of cancer responses to specific agents that contain enough information to make the evaluation possible (e.g., radiation-induced leukemia, breast and thyroid cancer, skin cancer induced by arsenic in drinking water, liver cancer induced by aflatoxins in the diet). Some supporting evidence also exists from animal experiments (e.g., the initiation stage of the two-stage carcinogenesis model

in rat liver and mouse skin).

Because its scientific basis, although limited, is the best of any of the current mathematical extrapolation models, the nonthreshold model, which is linear at low doses, has been adopted as the primary basis for risk extrapolation to low levels of the dose-response relationship. The risk estimates made with such a model should be regarded as conservative, representing a plausible upper limit for the risk; i.e., the true risk is not likely to be higher than the estimate, but it could be lower.

For several reasons, the unit risk estimate based on animal bioassays is only an approximate indication of the absolute risk in populations exposed to known carcinogen concentrations. First, there are important species differences in uptake, metabolism, and organ distribution of carcinogens, as well as species differences in target site susceptibility, immunological responses, hormone function, dietary factors, and disease. Second, the concept of equivalent doses for humans compared to animals on a mg/surface area basis is virtually without experimental verification as regards carcinogenic response. Finally, human populations are variable with respect to genetic constitution and diet, living environment, activity patterns, and other cultural factors.

The unit risk estimate can give a rough indication of the relative potency of a given agent as compared with other carcinogens. Such estimates are, of course, more reliable when the comparisons are based on studies in which the test species, strain, sex, and routes of exposure are similar.

The quantitative aspect of carcinogen risk assessment is addressed here because of its possible value in the regulatory decision-making process, e.g., in setting regulatory priorities, evaluating the adequacy of technology-based controls, etc. However, the imprecision of presently available technology for estimating cancer risks to humans at low levels of exposure should be recog-

nized. At best, the linear extrapolation model used here provides a rough but plausible estimate of the upper limit of risk--that is, with this model it is not likely that the true risk would be much more than the estimated risk, but it could be considerably lower. The risk estimates presented in subsequent sections should not be regarded, therefore, as accurate representations of the true cancer risks even when the exposures involved are accurately defined. The estimates presented may, however, be factored into regulatory decisions to the extent that the concept of upper-risk limits is found to be useful.

9.3.3.2 Mathematical Description of the Low-Dose Extrapolation Model--The mathematical formulation chosen to describe the linear nonthreshold dose-response relationship at low doses is the linearized multistage model. This model employs enough arbitrary constants to be able to fit almost any monotonically increasing dose-response data, and it incorporates a procedure for estimating the largest possible linear slope (in the 95% confidence limit sense) at low extrapolated doses that is consistent with the data at all dose levels of the experiment.

Let $P(d)$ represent the lifetime risk (probability) of cancer at dose d . The multistage model has the form

$$P(d) = 1 - \exp [-(q_0 + q_1d + q_1d^2 + \dots + q_kd^k)]$$

where

$$q_i \geq 0, i = 0, 1, 2, \dots, k$$

Equivalently,

$$P_t(d) = 1 - \exp [-(q_1d + q_2d^2 + \dots + q_kd^k)]$$

where

$$P_t(d) = \frac{P(d) - P(0)}{1 - P(0)}$$

is the extra risk over background rate at dose d or the effect of treatment.

The point estimate of the coefficients q_i , $i = 0, 1, 2, \dots, k$, and consequently, the extra risk function, $P_t(d)$, at any given dose d , is calculated by maximizing the likelihood function of the data.

The point estimate and the 95% upper confidence limit of the extra risk, $P_t(d)$, are calculated by using the computer program, GLOBAL83, developed by Howe (1983). At low doses, upper 95% confidence limits on the extra risk and lower 95% confidence limits on the dose producing a given risk are determined from a 95% upper confidence limit, q_1^* , on parameter q_1 . Whenever $q_1 > 0$, at low doses the extra risk $P_t(d)$ has approximately the form $P_t(d) = q_1 \times d$. Therefore, $q_1^* \times d$ is a 95% upper confidence limit on the extra risk and R/q_1^* is a 95% lower confidence limit on the dose, producing an extra risk of R . Let L_0 be the maximum value of the log-likelihood function. The upper-limit, q_1^* , is calculated by increasing q_1 to a value q_1^* such that when the log-likelihood is remaximized subject to this fixed value, q_1^* , for the linear coefficient, the resulting maximum value of the log-likelihood L_1 satisfies the equation

$$2 (L_0 - L_1) = 2.70554$$

where 2.70554 is the cumulative 90% point of the chi-square distribution with one degree of freedom, which corresponds to a 95% upper-limit (one-sided). This approach of computing the upper confidence limit for the extra risk $P_t(d)$ is an improvement on the Crump et al. (1977) model. The upper confidence limit for the extra risk calculated at low doses is always linear. This is conceptually consistent with the linear nonthreshold concept discussed earlier. The

slope, q_1^* , is taken as an upper bound of the potency of the chemical in inducing cancer at low doses. (In the section calculating the risk estimates, $P_t(d)$ will be abbreviated as P .)

In fitting the dose-response model, the number of terms in the polynomial is chosen equal to $(h-1)$, where h is the number of dose groups in the experiment, including the control group.

Whenever the multistage model does not fit the data sufficiently well, data at the highest dose is deleted and the model is refit to the rest of the data. This is continued until an acceptable fit to the data is obtained. To determine whether or not a fit is acceptable, the chi-square statistic

$$\chi^2 = \sum_{i=1}^h \frac{(X_i - N_i P_i)^2}{N_i P_i (1 - P_i)}$$

is calculated where N_i is the number of animals in the i^{th} dose group, X_i is the number of animals in the i^{th} dose group with a tumor response, P_i is the probability of a response in the i^{th} dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups. The fit is determined to be unacceptable whenever χ^2 is larger than the cumulative 99% point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of non-zero multistage coefficients.

9.3.3.3 Adjustments for Less Than Lifespan Duration of Experiment--If the duration of experiment L_e is less than the natural lifespan of the test animal L , the slope q_1^* , or more generally the exponent $g(d)$, is increased by multiplying a factor $(L/L_e)^3$. We assume that if the average dose d is continued, the age-specific rate of cancer will continue to increase as a constant function

of the background rate. The age-specific rates for humans increase at least by the third power of the age and often by a considerably higher power, as demonstrated by Doll (1971). Thus, it is expected that the cumulative tumor rate would increase by at least the third power of age. Using this fact, it is assumed that the slope q_1^* , or more generally the exponent $g(d)$, would also increase by at least the third power of age. As a result, if the slope q_1^* [or $g(d)$] is calculated at age L_e , it is expected that if the experiment had been continued for the full lifespan L at the given average exposure, the slope q_1^* [or $g(d)$] would have been increased by at least $(L/L_e)^3$.

This adjustment is conceptually consistent with the proportional hazard model proposed by Cox (1972) and the time-to-tumor model considered by Daffer et al. (1980), where the probability of cancer by age t and at dose d is given by

$$P(d,t) = 1 - \exp [-f(t) \times g(d)].$$

9.3.4 Unit Risk for a Compound

9.3.4.1 Definition of Unit Risk--This section deals with the unit risk for PCE in air and water and the potency of PCE relative to other carcinogens that the CAG has evaluated. The unit risk estimate for an air or water pollutant is defined as the incremental lifetime cancer risk occurring in a hypothetical population in which all individuals are exposed continuously from birth throughout their lifetimes to a concentration of $1 \mu\text{g}/\text{m}^3$ of the agent in the air they breathe, or to $1 \mu\text{g}/\text{L}$ in the water they drink. This calculation is done to estimate in quantitative terms the impact of the agent as a carcinogen. Unit risk estimates are used for two purposes: 1) to compare the carcinogenic potency of several agents with each other, and 2) to give a crude indication of the population risk which might be associated with air or water exposure to these agents, if the actual exposures are known.

For most cases of interest to risk assessment, the 95% upper-limit risk associated with dose units/day as obtained by means of the GLOBAL83 computer program (Howe, 1983), can be adequately approximated by $P(d) = 1 \exp [-(q_1^* d)]$.

9.3.4.2 Unit Risk Estimates and Interpretation--The unit risk estimate based on animal bioassays is only an approximate indication of the absolute risk in populations exposed to known carcinogen concentrations. There may be important differences in target site susceptibility, immunological responses, hormone function, dietary factors, and disease. In addition, human populations are variable with respect to genetic constitution and diet, living environment, activity patterns, and other cultural factors.

The unit risk estimate can give a rough indication of the relative potency of a given agent compared with other carcinogens. The comparative potency of different agents is more reliable when the comparison is based on studies in the same test species, strain, and sex.

The quantitative aspect of carcinogen risk assessment is included here because it may be of use in the regulatory decision-making process, e.g., setting regulatory priorities, evaluating the adequacy of technology-based controls, etc. However, it should be recognized that the estimation of cancer risks to humans at low levels of exposure is uncertain. At best, the linear extrapolation model used here provides a rough but plausible estimate of the upper-limit of risk; i.e., it is not likely that the true risk would be much more than the estimated risk, but it could very well be considerably lower. The risk estimates presented in subsequent sections should not be regarded as immutable representations of the true cancer risks; however, the estimates presented may be factored into regulatory decisions to the extent that the concept of upper risk limits is found to be useful. Table 9-10 gives the upper-limit slope estimates, q_1^* , from the linearized multistage model for PCE. The slope

estimate can be used to compare the relative carcinogenic potency of PCE to that of other potential human carcinogens. The slope is also used to calculate upper-bound incremental risks at low levels of exposure.

9.3.5 Alternative Low-Dose Extrapolation Models

In addition to the multistage model, two more models, referred to as the probit and the Weibull, are often employed by the CAG for low-dose extrapolation for purposes of comparison. However, calculations made with these two alternative models are not presented in this document, since no meaningful comparisons can be made on the basis of these calculations. For example, the 95% upper-bound estimate of cancer risk at the metabolized dose 0.1 mg/kg/day is predicted to be 1 by either the probit or the Weibull model, while the corresponding risk estimated by the multistage model is only 0.02.

9.4 CALCULATION OF UNIT RISK

9.4.1 Potency (Slope) Estimate on the Basis of Animal Data

The dose metabolized, which is defined as the total uptake minus the amount expired unchanged, is used as the dosimetry in the risk assessment. The cancer potency (slope) calculated on this basis can be converted back to the administered dose by using the dose-metabolism relationship estimated from experimental data.

Table 9-10 presents the carcinogenic potency for humans along with the metabolized doses calculated by two approaches and tumor incidence rates in the NCI (1977a) bioassay. The detailed derivations of the metabolized doses are presented in Tables 9-8 and 9-9. To account for the high mortality rates in the dosed groups, the animals that died before the occurrence of the first hepatocellular carcinomas are excluded from the denominators of tumor incidence rates. Another approach would be to use the time-to-death data for risk calculation. This approach yields a slightly higher risk value than that calculated

TABLE 9-10. DOSE-RESPONSE DATA AND POTENCY (SLOPE) ESTIMATES

Data base for calculating body burden	LAE ^a (animal) (mg/kg/day)	Tumor incidence ^b	Potency estimate ^c (mg/kg/day) ⁻¹	
			q ₁ [*] (M)	q ₁ [*] (A)
Method 1: Use total metabolized dose (Schumann et al., 1980)	<u>Male mice:</u>			
	0	2/20		
	56.41	32/48	1.8x10 ⁻¹	4.4x10 ⁻²
	112.82	27/45		
	<u>Female mice:</u>			
	0	0/20		
Method 2: Use dose metabolized in urinary excretion (Buben and O'Flaherty, 1985)	40.62	19/48	1.6x10 ⁻¹	4.0x10 ⁻²
	81.24	19/45		
	<u>Male mice:</u>			
	0	2/20		
	37.73	32/48	3.4x10 ⁻¹	6.8x10 ⁻²
	52.11	27/45		
	<u>Female mice:</u>			
	0	0/20		
	31.07	19/48	2.5x10 ⁻¹	5.1x10 ⁻²
	45.39	19/45		

^aSee Tables 9-8 and 9-9 for the animal lifetime average exposures (LAE).

^bThe denominators are the number of animals that survived at the time the first hepatocellular carcinoma occurred in each study. The first tumor occurred at 27 weeks for the male mice and at 41 weeks for the female mice.

^cq₁^{*}(M) is the potency estimate expressed in terms of the metabolized dose.

q₁^{*}(A) is the potency estimate expressed in terms of the administered dose.

on the basis of incidence data with a modified denominator. However, the risk calculated from the time-to-death data is difficult to interpret because the majority of animals with tumors were discovered at the terminal sacrifice. Therefore, only the potency estimates calculated on the basis of incidence data are presented.

Two sets of potency estimates are presented in the last two columns of Table 9-10, using either the total dose metabolized (Method 1) or the dose metabolized and eliminated in urine (Method 2). These estimates are calculated by the linearized multistage model, using the dose-response data in Table 9-10. The values $q_1^*(M)$ and $q_1^*(A)$ are potency estimates expressed, respectively, in terms of the metabolized and the administered doses.

The carcinogenic potency $q_1^*(M)$ for humans is calculated by multiplying the animal potency $q_1^*(M)$ by a factor $(W_H/W_A)^{1/3}$, where W_H and W_A are, respectively, the body weights for humans and animals. The underlying assumption for this adjustment is that the metabolized dose per surface area is equally effective in inducing cancer among species. To convert from $q_1^*(M)$ to $q_1^*(A)$, the relationship between administered dose (A) and the amount metabolized (M) is utilized. For the estimates calculated according to Method 2, the relationship $M = 0.20A$ is used. This relationship can be obtained from the dose-metabolism relationship derived by Buben and O'Flaherty (1985) when the administered dose A is small. For the estimates calculated by Method 1, the relationship $M = 0.25A$ is used, as in Buben and O'Flaherty (1985), and considering the fact that the urinary metabolites account for about 80% of the total metabolites. It is worthwhile to note that these relationships are consistent with the empirical observations of Pegg et al. (1979), in which approximately 29% of the dose was metabolized after rats were given a small dose (1 mg/kg or 0.25 mg per animal).

Because the body burdens calculated by Methods 1 and 2 result in compa-

rable potency estimates, and because the available human body burden via inhalation is given in terms of urinary metabolites, it is recommended that the potency estimates on the basis of Method 2 be used to represent the carcinogenic potency of PCE. Since more reliable dose-response data exist for female mice than for male mice, the carcinogenic potency calculated from these data is used. The slope, q_1^* , estimate for PCE is:

$$q_1^*(A) = 5.1 \times 10^{-2} \text{ mg/kg/day}$$

expressed in terms of the administered dose, and

$$q_1^*(M) = 2.5 \times 10^{-1} \text{ mg/kg/day}$$

expressed in terms of the metabolized dose in urinary excretion.

9.4.2 Risk Associated with 1 $\mu\text{g/L}$ of PCE in Drinking Water

The daily PCE intake (mg/kg/day) for a 70 kg person consuming 2 L of water contaminated with 1 $\mu\text{g/L}$ of PCE is:

$$\begin{aligned} d &= (1 \mu\text{g/L}) \times (2 \text{ L/day}) \times (10^{-3} \text{ mg}/\mu\text{g})/70 \text{ kg} \\ &= 2.9 \times 10^{-5} \text{ mg/kg/day} \end{aligned}$$

Therefore, the upper-bound estimate of the incremental lifetime risk due to consuming water contaminated with 1 $\mu\text{g/L}$ of PCE is:

$$P = 5.1 \times 10^{-2} \times 2.9 \times 10^{-5} = 1.5 \times 10^{-6}$$

9.4.3 Risk Associated with 1 $\mu\text{g}/\text{m}^3$ of PCE in Air

In order to estimate the risk due to 1 $\mu\text{g}/\text{m}^3$ of PCE in air, it is necessary to estimate the amount metabolized that accompanies exposure to this concentration in air. For PCE, the body burden can be estimated from both human

and animal data. Since only the metabolized dose in urine is available for humans, the potency $q_1^*(M) = 2.5 \times 10^{-1}$ mg/kg/day in terms of urinary metabolites will be used in the risk calculation.

9.4.3.1 Unit Risk Calculated on the Basis of Body Burden Derived from Human Data--In an experimental human exposure to PCE vapor, Fernandez et al. (1976) observed that in 72 hours, the quantity of TCA metabolized and eliminated in urine was about 25 mg following 150 ppm exposure for 8 hours. Assuming that the amount metabolized in urine is linearly proportional both to the air concentration and to the duration of exposure, the observations of Fernandez et al. (1976) imply that a continuous (24 hours) exposure to 1 ppm of PCE produces $25 \times 3/150 = 0.5$ mg/day of urinary metabolites. Since $1 \text{ ppm} = 6,908 \mu\text{g}/\text{m}^3$, a continuous exposure to $1 \mu\text{g}/\text{m}^3$ of PCE yields a metabolized dose of 7.3×10^{-5} mg/day. For chronic and continuous exposures, the metabolized dose would be $7.3 \times 10^{-5}/70 = 1.0 \times 10^{-6}$ mg/kg/day. Therefore, the incremental lifetime risk due to $1 \mu\text{g}/\text{m}^3$ of PCE in air is:

$$P = 2.5 \times 10^{-1} \times 1.0 \times 10^{-6} = 2.5 \times 10^{-7}$$

A study by Bolanowska and Golacka (1972) also provides some information on the relationship between the air concentration and the amount metabolized in urinary excretion. In this study, five subjects were exposed to $390,000 \mu\text{g}/\text{m}^3$ of PCE (approximately 50 ppm) for 6 hours. The concentration of metabolites in urine (mg/hr) is presented graphically over the 20-hour period during and after exposure. The total amount of metabolites in urine (area under curve) is estimated to be about 13 mg. The area under the curve from 20 hours to infinity is calculated by $C \times t_{1/2}/0.693$, where C is the concentration at the last sampling time (i.e., 20 hours) and $t_{1/2}$, the half-life, is assumed to be 100 hours. Under the assumption that the amount metabolized is linearly

proportional to the air concentration and the duration of exposure, the amount metabolized, associated with $1 \mu\text{g}/\text{m}^3$ of PCE in air, is

$$(13 \text{ mg}/390,000) \times (24 \text{ hours}/6 \text{ hours}) = 1.33 \times 10^{-4} \text{ mg/day}$$

Equivalently, the amount metabolized due to continuous exposure to $1 \mu\text{g}/\text{m}^3$ of PCE in air would be $1.33 \times 10^{-4} \text{ mg/day}$ for a 70-kg man, which would be $1.9 \times 10^{-6} \text{ mg/kg/day}$. Thus, the cancer risk due to $1 \mu\text{g}/\text{m}^3$ of PCE in air is estimated to be

$$P = 2.5 \times 10^{-1} \times 1.9 \times 10^{-6} = 4.8 \times 10^{-7}$$

This risk estimate is about two times higher than that calculated on the basis of Fernandez et al. (1976). Since the study by Bolanowska and Golacka (1972) was conducted at lower concentrations, the risk calculation on the basis of this study is preferred. It is therefore recommended that 4.8×10^{-7} be used as the unit risk estimate for PCE in air.

9.4.3.2 Unit Risk Calculated on the Basis of Body Burden Derived from Animal Data--Table 9-6 contains some animal data that can be used to infer human metabolized dose when exposed to $1 \mu\text{g}/\text{m}^3$ of PCE in air. The dispositions of ^{14}C -PCE radioactivity in rats and mice 72 hours after exposure to 10 ppm of PCE in air for a period of 6 hours are as follows:

	Rats (mg/animal)	Mice (mg/animal)
Expired unchanged	1.008	0.048
Total metabolized	0.467 (32%)	0.356 (88%)
Metabolites in urinary excretion	0.275 (19%)	0.285 (71%)
Total uptake	1.475	0.404

These data indicate that the percentage of PCE that is metabolized decreases with an increase of body weight and that the total uptake, not the amount metabolized, is approximately proportional to the body surface area between the two species. To estimate the amount metabolized in human urinary excretion after PCE exposure, it is assumed that the total uptake is proportional to the body surface area among species, including humans, and the relationship between the percentage metabolized (M) and the body weight (W) follows the equation $M = AW^K$. The parameters A and K are estimated to be $A = 0.146$ and $K = 0.420$, using the animal data presented above. Thus, for a 70-kg person, the percentage metabolized is predicted to be 2.5%. This is consistent with the empirical observation in humans (Monster, 1979). On this basis, if humans were exposed to 10 ppm of PCE in air, as were the animals, the amount metabolized would be

$$1.48 \times (70/0.25)^{2/3} \times 0.025 = 1.58 \text{ mg}$$

where $1.48 \times (70/0.25)^{2/3}$ is the estimated total uptake using the surface area correction factor $(70/0.25)^{2/3}$.

Under the assumption that the amount metabolized (in urine) is linearly proportional to both the air concentration and the duration of exposure, the amount metabolized due to a continuous (24-hour) exposure to 1 ppm of PCE in air is $1.58 \times 4/10 = 0.63 \text{ mg/day}$. Equivalently, the body metabolic load is $0.63/6,908 = 9.2 \times 10^{-5} \text{ mg}$ for a 24-hour exposure when the air concentration is $1 \mu\text{g}/\text{m}^3$, using the fact that $1 \text{ ppm} = 6,908 \mu\text{g}/\text{m}^3$. For chronic and continuous exposure, the amount metabolized per day due to $1 \mu\text{g}/\text{m}^3$ of PCE in air is $9.2 \times 10^{-5} \text{ mg}/70 \text{ kg/day} = 1.3 \times 10^{-6} \text{ mg/kg/day}$. Therefore, the incremental lifetime risk due to $1 \mu\text{g}/\text{m}^3$ of PCE is:

$$P = 2.5 \times 10^{-1} \times 1.3 \times 10^{-6} = 3.3 \times 10^{-7}$$

This estimate is about the same as that calculated on the basis of direct observations regarding human metabolized dose. Since data based on direct observation of humans is preferred to the animal data, it is recommended that the value 4.8×10^{-7} , calculated on the basis of actual human metabolized dose, be used as the unit risk estimate for PCE in air.

9.4.3.3 Unit Risk Calculated on the Basis of a Negative Inhalation Study--

For comparison, a negative inhalation study on rats, sponsored by the Dow Chemical Company (Rampy et al., 1978) is used to calculate an upper-bound estimate of risk for PCE. Ninety-four animals of each sex were exposed to either 300 ppm or 600 ppm of PCE, 6 hours/day, 5 days/week for 52 weeks. Animals were allowed to survive until the terminal sacrifice at 31 months. A shortcoming of this study was that the animals were exposed to PCE for only 52 weeks. The use of data from such a study may seriously underestimate the risk. Among the four PCE-exposed groups, the data from the female high-dose group yield the smallest upper-bound estimate of risk, and thus will be used to calculate the unit risk for PCE in air. Forty-nine female rats in the high-dose (600 ppm) group survived beyond 2 years. The 95% upper-bound estimate of the probability of cancer among these 49 animals with no cancer response is $P_u = 0.059$. The corresponding lifetime average exposure is

$$d = 600 \text{ ppm} \times \frac{5 \text{ days}}{7 \text{ days}} \times \frac{6 \text{ hours}}{24 \text{ hours}} \times \frac{52 \text{ weeks}}{104 \text{ weeks}} = 53.57 \text{ ppm}$$

or

$$d = 370,071 \mu\text{g}/\text{m}^3$$

Thus, the carcinogenic potency of PCE, defined as the slope in the one-hit model, is calculated to be

$$b = [-\ln(1-P_U)]/d = 1.6 \times 10^{-7}/\mu\text{g}/\text{m}^3$$

for animals.

To calculate the risk to humans associated with $1 \mu\text{g}/\text{m}^3$ of PCE in air, it is necessary to determine the human exposure concentration $C(\mu\text{g}/\text{m}^3)$ equivalent to $1 \mu\text{g}/\text{m}^3$ of PCE in air for rats. Assuming that dose in relation to surface area is equally effective among species, the human exposure concentration $C(\mu\text{g}/\text{m}^3)$ satisfies the equation

$$\begin{aligned} C(\mu\text{g}/\text{m}^3) \times (20 \text{ m}^3/\text{day})/(70 \text{ kg})^{2/3} \\ = 1 \mu\text{g}/\text{m}^3 \times (0.224 \text{ m}^3/\text{day})/(0.35 \text{ kg})^{2/3} \end{aligned}$$

where $20 \text{ m}^3/\text{day}$ and $0.224 \text{ m}^3/\text{day}$ are the daily air intakes, respectively, for a 70-kg person and an 0.35-kg rat. Thus, $C(\mu\text{g}/\text{m}^3) = 0.39 \mu\text{g}/\text{m}^3$. Therefore, the human risk due to $1 \mu\text{g}/\text{m}^3$ of PCE in air is

$$P = 1.6 \times 10^{-7}/0.39 = 4.1 \times 10^{-7}$$

This risk estimate is again comparable to those calculated previously.

9.4.3.4 Discussion

To the extent possible, the available metabolism and pharmacokinetic information for PCE has been used in the risk calculations. Nevertheless, the use of pharmacokinetic information may reduce some of the ambiguity associated with three major areas of risk assessment uncertainty listed below:

1. Extrapolation from high dose-response in animals to low dose-response

- in animals (i.e., low-dose extrapolation),
2. Extrapolation from low dose-response in animals to low dose-response in humans (i.e., species conversion), and
 3. Extrapolation from gavage to inhalation (i.e., route-to-route extrapolation).

In calculating the dose-response relationship for PCE, the amount metabolized is considered to be an effective dose. The use of this surrogate effective dose may not eliminate the uncertainty associated with the low-dose extrapolation because the dose actually reaching the receptor sites may not be linearly proportional to the total amount metabolized, and the shape of the dose-response relationship is still unknown. However, it seems reasonable to expect that the uncertainty with regard to the low-dose extrapolation would be somewhat reduced by the use of the metabolized dose because the metabolized dose better reflects the dose-response relationship, particularly in the high-dose region.

To extrapolate from animals to humans, the metabolized dose per body surface area is assumed to be equivalent (i.e., equally potent) among species. This assumption is by no means supported by the empirical data. An alternative approach for animal-to-human extrapolation would be to assume that mg metabolized dose/kg/day is equivalent among species. If this assumption is made, the slope factor q_1^* , as calculated for PCE and expressed in terms of $(\text{mg/kg/day})^{-1}$, would be reduced by approximately 10 times.

The carcinogenic potency calculated from the gavage study and on the basis of metabolized dose does provide a better basis for extrapolating the risk estimate from gavage to inhalation. This is so because the relationship between the ambient air concentration and the amount metabolized for PCE is available

for both humans and animals. Therefore, the uncertainty due to route-to-route extrapolation should be greatly reduced by the use of metabolized dose.

9.5 RISK PREDICTION AGAINST HUMAN EXPERIENCE

Although there is no sound epidemiologic data that could be used to estimate the carcinogenic potential of PCE, efforts are made herein to determine whether or not the risk estimate of 4.8×10^{-7} per $\mu\text{g}/\text{m}^3$ is reasonable in view of the fact that none of the epidemiologic studies on dry-cleaners showed significant elevation of cancer risk. The major human experience in PCE exposure has been on the part of dry-cleaning workers. Among them, the machine operators are the workers most heavily exposed, with a time-weighted average concentration of about 22 ppm. For other dry-cleaners, the mean exposure concentration is approximately 3 ppm (Ludwig et al., 1983).

According to Campbell et al. (1980), the number of persons employed as machine operators in commercial, industrial, and coin-operated dry-cleaning facilities in the United States is estimated to be 27,700. A question to be asked is whether a significant increase of cancer deaths should be expected among dry-cleaning workers, and in particular, among the machine operators. As demonstrated below, a cohort study on dry-cleaning workers is unlikely to show an elevation of cancer risk.

At an ambient air concentration of 22 ppm (for machine operators) and 3 ppm (for other dry-cleaners), workers who work 8 hours/day, 250 days/year for one-third of their lifetime would have an average daily exposure of

$$\begin{aligned} d &= 22 \text{ ppm} \times 6908 (\mu\text{g}/\text{m}^3)/\text{ppm} \times \frac{8 \text{ hours}}{24 \text{ hours}} \times \frac{250 \text{ days}}{365 \text{ days}} \times \frac{1}{3} \\ &= 11,565.90 \mu\text{g}/\text{m}^3 \end{aligned}$$

for machine operators and, similarly,

$$d = 1577.17 \mu\text{g}/\text{m}^3$$

for other dry-cleaners. Therefore, the incremental lifetime risk is

$$P = 4.8 \times 10^{-7} \times 11,565.9 = 5.6 \times 10^{-3}$$

for machine operators, and

$$P = 4.8 \times 10^{-7} \times 1577.17 = 7.6 \times 10^{-4}$$

for other dry-cleaners.

The incremental lifetime risks for machine operators (5.6×10^{-3}) and other workers (7.6×10^{-4}) are not likely to be detected by a cohort study because of the large sample sizes required. The sample sizes required to show a significant elevation of cancer risk at a level of 0.05 (one-sided test) and a power of 0.80 are as follows, using the formula presented in Schlesselman (1974).

Background lifetime cancer rates	Sample size required	
	Machine operators	Other workers
0.01	5,000	218,000
0.05	25,000	509,000
0.10	36,263	1,705,000

The sample size required depends on the background cancer rate. Since no specific cancers are known to be associated with PCE exposure, three hypothetical background rates are used in the calculations, which should cover most of the rates for specific cancers in the U.S. population.

9.6 COMPARISON OF POTENCY WITH OTHER COMPOUNDS

One of the uses of the concept of unit risk is to compare the relative potencies of carcinogens. Figure 9-6 is a histogram representing the frequency distribution of potency indices for 54 suspect carcinogens evaluated by the CAG. The actual data summarized by the histogram are presented in Table 9-11. The potency index is derived from q_1^* , the 95% upper bound of the linear component in the multistage model, and is expressed in terms of $(\text{mmol/kg/day})^{-1}$. Where human data are available for an agent, they have been used to calculate the index. Where no human data are available, animal oral studies are used in preference to animal inhalation studies, since oral studies have constituted the majority of animal studies.

Based on available data concerning hepatocellular carcinomas in female mice, the potency index for PCE has been calculated as 8×10^0 . This figure is derived by multiplying the slope $q_1^* = 5.1 \times 10^{-2} \text{ (mg/kg/day)}$ and the molecular weight of PCE, 165.8. This places the potency index for PCE in the fourth quartile of the 54 suspect carcinogens evaluated by the CAG.

The ranking of relative potency indices is subject to the uncertainties involved in comparing a number of potency estimates for different chemicals based on varying routes of exposure in different species, by means of data from studies whose quality varies widely. All of the indices presented are based on estimates of low-dose risk, using a low-dose linear extrapolation model. These indices may not be appropriate for the comparison of potencies if linearity does not exist at the low-dose range, or if comparison is to be made at the high-dose range. If the latter is the case, then an index other than the one calculated may be more appropriate.

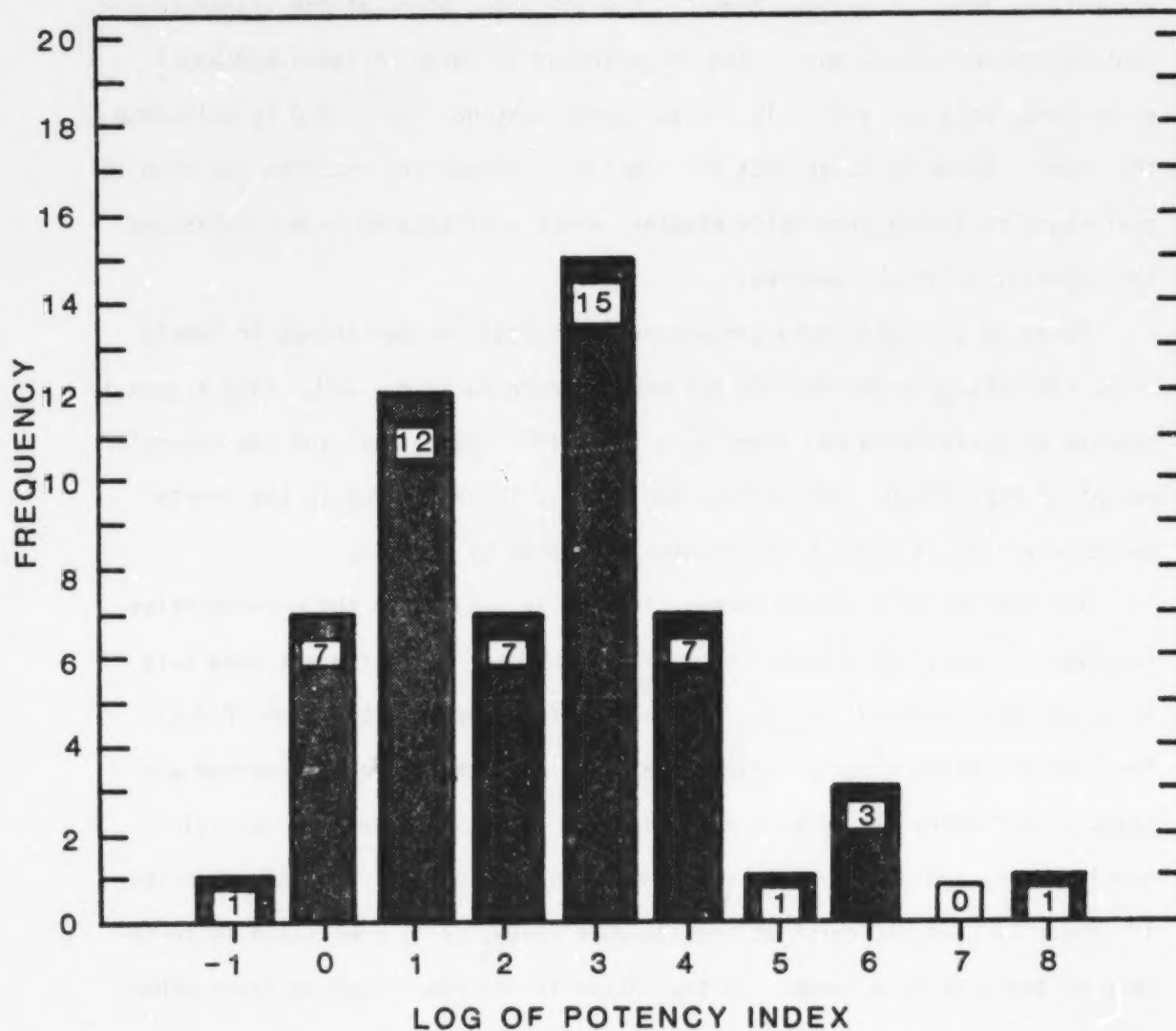
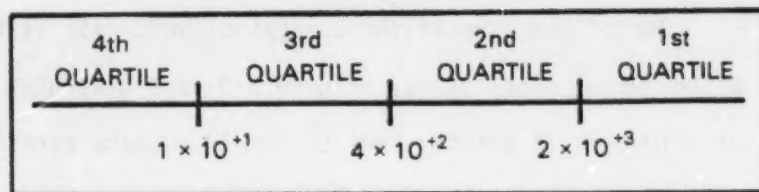


Figure 9-6. Histogram representing the frequency distribution of the potency indices of 54 suspect carcinogens evaluated by the Carcinogen Assessment Group.

TABLE 9-11. RELATIVE CARCINOGENIC POTENCIES AMONG 54 CHEMICALS EVALUATED BY THE CARCINOGEN ASSESSMENT GROUP AS SUSPECT HUMAN CARCINOGENS

Compounds	CAS Number	Level of evidence ^a		Grouping based on IARC criteria	Slope ^b (mg/kg/day) ⁻¹	Molecular weight	Potency index ^c	Order of magnitude (log ₁₀ index)
Acrylonitrile	107-13-1	L	S	2A	0.24(W)	53.1	1x10 ⁺¹	+1
Aflatoxin B ₁	1162-65-8	L	S	2A	2900	312.3	9x10 ⁺⁵	+6
Aldrin	309-00-2	I	L	2B	11.4	369.4	4x10 ⁺³	+4
Allyl chloride	107-05-1				1.19x10 ⁻²	76.5	9x10 ⁻¹	0
Arsenic	7440-38-2	S	I	1	15(H)	149.8	2x10 ⁺³	+3
B[a]P	50-32-8	I	S	2B	11.5	252.3	3x10 ⁺³	+3
Benzene	71-43-2	S	S	1	2.9x10 ⁻² (W)	78	2x10 ⁰	0
Benzidene	92-87-5	S	S	1	234(W)	184.2	4x10 ⁺⁴	+5
Beryllium	7440-41-7	L	S	2A	2.6	9	2x10 ⁺¹	+1
1,3-Butadiene	106-99-0	I	S	2B	1.0x10 ⁻¹ (I)	54.1	5x10 ⁰	+1
Cadmium	7440-43-9	L	S	2A	6.1(W)	112.4	7x10 ⁺²	+3
Carbon tetrachloride	56-23-5	I	S	2B	1.30x10 ⁻¹	153.8	2x10 ⁺¹	+1
Chlordane	57-74-9	I	L	3	1.61	409.8	7x10 ⁺²	+3

(continued on the following page)

TABLE 9-11. (continued)

Compounds	CAS Number	Level of evidence ^a		Grouping based on IARC criteria	Slope ^b (mg/kg/day) ⁻¹	Molecular weight	Potency index ^c	Order of magnitude (log ₁₀ index)
		Humans	Animals					
Chlorinated ethanes								
1,2-Dichloroethane	107-06-2	I	S	28	9.1x10 ⁻²	98.9	9x10 ⁰	+1
hexachloroethane	67-72-1	I	L	3	1.42x10 ⁻²	236.7	3x10 ⁰	0
1,1,2,2-Tetrachloroethane	79-34-5	I	L	3	0.20	167.9	3x10 ⁺¹	+1
1,1,2-Trichloroethane	79-00-5	I	L	3	5.73x10 ⁻²	133.4	8x10 ⁰	+1
Chloroform	67-66-3	I	S	28	7x10 ⁻²	119.4	8x10 ⁰	+1
Chromium VI	7440-47-3	S	S	1	41(W)	100	4x10 ⁺³	+4
DDT	50-29-3	I	S	28	0.34	354.5	1x10 ⁺²	+2
Dichlorobenzidine	91-94-1	I	S	28	1.69	253.1	4x10 ⁺²	+3
1,1-Dichloroethylene (Vinylidene chloride)	75-35-4	I	L	3	1.16(I)	97	1x10 ⁺²	+2
Dichloromethane (Methylene chloride)	75-09-2	I	L	3	6.3x10 ⁻⁴ (I)	84.9	5x10 ⁻²	-1
Dieldrin	60-57-1	I	S	28	30.4	380.9	1x10 ⁺⁴	+4
2,4-Dinitrotoluene	121-14-2	I	S	28	0.31	182	6x10 ⁺¹	+2
Diphenylhydrazine	122-66-7	I	S	28	0.77	180	1x10 ⁺²	+2
Epichlorohydrin	106-89-8	I	S	28	9.9x10 ⁻³	92.5	9x10 ⁻¹	0
Bis(2-chloroethyl)ether	111-44-4	I	S	28	1.14	143	2x10 ⁺²	+2

(continued on the following page)

TABLE 9-11. (continued)

Compounds	CAS Number	Level of evidence ^a		Grouping based on IARC criteria	Slope ^b (mg/kg/day) ⁻¹	Molecular weight	Potency index ^c	Order of magnitude (log ₁₀ index)
		Humans	Animals					
Bis(chloromethyl) ether	542-88-1	S	S	1	9300(I)	115	1x10 ⁺⁶	+6
Ethylene dibromide (EDB)	106-93-4	I	S	2B	41	187.9	8x10 ⁺³	+4
Ethylene oxide	75-21-8	L	S	2A	3.5x10 ⁻¹ (I)	44.1	2x10 ⁺¹	+1
Heptachlor	76-44-8	I	S	2B	3.37	373.3	1x10 ⁺³	+3
Hexachlorobenzene	118-74-1	I	S	2B	1.67	284.4	5x10 ⁺²	+3
Hexachlorobutadiene	87-68-3	I	L	3	7.75x10 ⁻²	261	2x10 ⁺¹	+1
Hexachlorocyclohexane technical grade					4.75	290.9	1x10 ⁺³	+3
alpha isomer	319-84-6	I	S	2B	11.12	290.9	3x10 ⁺³	+3
beta isomer	319-85-7	I	L	3	1.84	290.9	5x10 ⁺²	+3
gamma isomer	58-89-9	I	L	2B	1.33	290.9	4x10 ⁺²	+3
Hexachlorodibenzodioxin	34465-46-8	I	S	2B	6.2x10 ⁺³	391	2x10 ⁺⁶	+6
Nickel	7440-02-0	L	S	2A	1.15(W)	58.7	7x10 ⁺¹	+2
Nitrosamines								
Dimethylnitrosamine	62-75-9	I	S	2B	25.9(not by qt)	74.1	2x10 ⁺³	+3
Diethylnitrosamine	55-18-5	I	S	2B	43.5(not by qt)	102.1	4x10 ⁺³	+4
Dibutylnitrosamine	924-16-3	I	S	2B	5.43	158.2	9x10 ⁺²	+3
N-nitrosopyrrolidine	930-55-2	I	S	2B	2.13	100.2	2x10 ⁺²	+2
N-nitroso-N-ethylurea	759-73-9	I	S	2B	32.9	117.1	4x10 ⁺³	+4

(continued on the following page)

TABLE 9-11. (continued)

Compounds	CAS Number	Level of evidence ^a Humans Animals	Grouping based on IARC criteria	Slope ^b (mg/kg/day) ⁻¹	Molecular weight	Potency index ^c	Order of magnitude (log ₁₀ index)
N-nitroso-N-methylurea	684-93-5	I	S	302.6	103.1	3x10 ⁺⁴	+4
N-nitroso-diphenylamine	86-30-6	I	S	4.92x10 ⁻³	198	1x10 ⁰	0
PCBs	1336-36-3	I	S	4.34	324	1x10 ⁺³	+3
Phenols							
2,4,6-Trichlorophenol	88-06-2	I	S	1.99x10 ⁻²	197.4	4x10 ⁰	+1
Tetrachlorodibenzo- p-dioxin (TCDD)	1746-01-6	I	S	1.56x10 ⁺⁵	322	5x10 ⁺⁷	+8
Tetrachloroethylene	127-18-4	I	L	5.1x10 ⁻²	165.8	8x10 ⁰	+1
Toxaphene	8001-35-2	I	S	1.13	414	5x10 ⁺²	+3
Trichloroethylene	79-01-6	I	L/S	1.1x10 ⁻²	131.4	1x10 ⁰	0
Vinyl chloride	75-01-4	S	S	1.75x10 ⁻² (I)	62.5	1x10 ⁰	0

^aS = Sufficient evidence; L = Limited evidence; I = Inadequate evidence.

^bAnimal slopes are 95% upper-bound slopes based on the linearized multistage model. They are calculated based on animal oral studies, except for those indicated by I (animal inhalation), W (human occupational exposure), and H (human drinking water exposure). Human slopes are point estimates based on the linear nonthreshold model. Not all of the carcinogenic potencies presented in this table represent the same degree of certainty. All are subject to change as new evidence becomes available. The slope value is an upper bound in the sense that the true value (which is unknown) is not likely to exceed the upper bound and may be much lower, with a lower bound approaching zero. Thus, the use of the slope estimate in risk evaluations requires an appreciation for the implication of the upper bound concept as well as the "weight of evidence" for the likelihood that the substance is a human carcinogen. ^cThe potency index is a rounded-off slope in (mmol/kg/day)⁻¹ and is calculated by multiplying the slopes in (mg/kg/day)⁻¹ by the molecular weight of the compound.

9.7 SUMMARY

9.7.1 Qualitative

Tetrachloroethylene (PCE) induced a statistically significant increase in the incidence of hepatocellular carcinomas in both high- and low-dose male and female B6C3F1 mice when administered by gavage for a period of 78 weeks. The PCE used was over 99% pure, but was estimated to contain epichlorohydrin concentrations of less than 500 ppm. It is unlikely that this response could be attributed to the low concentration of epichlorohydrin.

No carcinogenic effects were observed in lifetime studies of Osborne-Mendel rats given PCE by gavage or in Sprague-Dawley rats exposed via inhalation for 12 months followed by 12 months of observation. However, because of excessive dose-related mortality in the gavage experiment, and because of the low dose level in the inhalation study, no conclusions can be made about the carcinogenicity of PCE in rats.

Intraperitoneal injection of PCE in strain A mice induced no statistically significant incidence of pulmonary adenomas. In mouse skin initiation-promotion experiments, PCE did not initiate skin tumors, nor did it induce skin tumors when applied alone three times per week for the lifetime of the animals. However, because of inherent limitations in these assays, these negative results do not detract from the positive findings of the National Cancer Institute (NCI) mouse experiment.

In a cohort study of dry-cleaning workers exposed to PCE, it was found that workers were at an elevated risk of colon cancer mortality; however, as many as one half of these workers may have been exposed to petroleum distillates in their working history. Other epidemiologic studies that found an association between cancer risk and employment in the dry-cleaning industry, did not identify which dry-cleaning solvents the employees had been exposed to.

9.7.2 Quantitative

Data on hepatocellular carcinomas in male and female mice from an NCI (1977a) gavage study on PCE have been used to calculate the unit risk for water and air. The unit risk is defined as the increased lifetime cancer risk due to exposure to one unit of dose in drinking water ($1 \mu\text{g/L}$) or in air ($1 \mu\text{g/m}^3$). Evaluation of the metabolism data in mice, rats, and humans shows that common principal end metabolites have been identified and that there is no comparative experimental evidence that the metabolic pathways for PCE qualitatively differ for these species. The use of relevant metabolism and pharmacokinetic data in the development of unit risk calculations helps to minimize some of the uncertainty associated with route to route extrapolation.

The upper-bound estimate of the incremental cancer risk due to $1 \mu\text{g/L}$ of PCE in drinking water is 1.5×10^{-6} . The upper-bound estimate of the incremental cancer risk due to $1 \mu\text{g/m}^3$ of PCE in air is 4.8×10^{-7} . The fact that the weight-of-evidence for the carcinogenicity of PCE is limited should be taken into consideration in the use of these estimates.

None of the epidemiologic studies reviewed in this report are adequate for use in estimating unit risks. If PCE does cause cancer in humans, it is unlikely that a cohort study on dry cleaners would reveal this fact, since the incremental lifetime risk to this limited number of workers is too small to be detected.

The potency index for PCE is 8×10^0 , ranking it in the lowest quartile of the 54 chemicals that the CAG has evaluated as suspect carcinogens. This potency index is calculated on the basis of oral exposures.

9.8 CONCLUSIONS

PCE has induced malignant tumors of the liver in both male and female B6C3F1 mice. This constitutes a signal that PCE might be a carcinogen for humans. The technical adequacy and the strong nature of the positive response in the 1977 NCI bioassay makes it likely that a repeat bioassay would also be positive. In fact, the recent NTP study, currently under audit, showed similar positive results. This study, if validated, would influence the evidence for carcinogenicity of PCE.

According to the Agency's Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1984), the evidence for PCE carcinogenicity in animals is limited, and the epidemiologic data is inconclusive. The overall weight-of-evidence for the human carcinogenic potential of PCE is Group C, i.e., a possible human carcinogen.

According to a literal interpretation of the criteria of the International Agency for Research on Cancer (IARC), the animal data supporting the carcinogenicity of PCE would be classified as limited. Also, since existing epidemiologic data for PCE are inconclusive, its overall IARC ranking would be classified as Group 3, meaning that the data are inadequate, and PCE cannot be classified as to its human carcinogenicity.

While the weight of evidence is limited for PCE carcinogenicity (a possible human carcinogen by proposed EPA criteria and inadequate data for assessing carcinogenicity by IARC criteria), an upper-bound incremental unit risk has been estimated for both ingestion and inhalation exposure. This has been done to help answer the "what-if" question--if PCE is carcinogenic in humans what is the possible impact upon public health. The upper-bound incremental unit risk for inhalation of $1 \mu\text{g PCE}/\text{m}^3$ is 4.8×10^{-7} and the

unit risk for ingestion of 1 μg of PCE/L in drinking water is 1.5×10^{-6} .

The potency index for PCE, based on NCI gavage data, places it in the lowest quartile of the 54 chemicals that the CAG has evaluated as suspect or known carcinogens.

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15. SUPPLEMENTARY NOTES		
16. ABSTRACT Tetrachloroethylene (PCE) is a volatile solvent with important commercial applications. It has been detected in the ambient air of a variety of urban and nonurban areas of the United States. It has less frequently been detected in water but has been monitored generally at levels of 1 ppb or less. The lowest-observed-adverse-effect-level, based on central nervous system dysfunction, is about 100 ppm (678 mg/m ³). Toxicity testing in experimental animals, coupled with limited human data, suggests that long-term exposure of humans to ambient levels of PCE is not likely to represent a health concern. At the current time, the teratogenic potential of PCE for humans is unknown; the mammalian animal tests performed to date do not indicate any significant teratogenic potential. Although PCE epoxide has been found to be positive with respect to mutagenicity in bacterial systems, the data on pure PCE suggest that if it is mutagenic, it is a weak mutagen. PCE has been demonstrated to induce malignant tumors of the liver in mice. This constitutes limited evidence that PCE may be carcinogenic in humans. Because existing epidemiologic data for PCE is inconclusive, the overall ranking according to the Agency's Proposed Guidelines for Carcinogen Risk Assessment would suggest that PCE is possibly carcinogenic in humans.		
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